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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: PROMEXAL X50 Preservative. Registration Non-Food/Feed Use.
PC-Code 107107
Submission No. S475970
MRID Nos. 432250-02 and 431387-14 through 431387-34
Case No. 040889 (Registration)
Action No. 115 Non-Food/Feed Use
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ID No. 010182-GIL

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ACTION:

Zeneca, Inc. has applied for Registration of PROMEXAL X50 Preservative. PROMEXAL X50 Preservative is an aqueous solution containing the new chemical 2-methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI) as its active ingredient.

TB-II has been asked to review the toxicology studies submitted in support of the Registration application.

CONCLUSIONS:

1. The submitted toxicity studies have been reviewed, the classification of each study is given below.

2. Acute inhalation toxicity studies (Guideline 81-3) were not available for review. Due to the possible use of PROMEXAL X50 in emulsion paints (which may result in an aerosol/particulate exposure), acute inhalation toxicity studies (for the EP and the TGAI) are required. Acute inhalation studies (for the EP and the TGAI) are required if the product consists of, or under conditions of use will result in an inhalable material (e.g. gas, volatile substances, or aerosol/particulate).

3. The active ingredient MTI was found to be positive in an in vitro mutagenicity assay (cultured human lymphocytes/aberrations) and weakly positive in an in vivo mutagenicity assay (mouse micronucleus assay). Thus, an additional, follow-up mutagenicity study (a dominant lethal assay) is requested.

4. It is noted that both MTI and PROMEXAL X50 Preservative were placed in Toxicity Category I for primary eye irritation. The proposed signal word "WARNING" in the label for PROMEXAL X50 Preservative should be replaced by the signal word "DANGER", to conform with the Toxicity Category of I for the chemical [40 CFR §158.10 (h)].

DETAILED CONSIDERATIONS:

I. Background.

Zeneca, Inc. has applied for Registration of PROMEXAL X50 Preservative. PROMEXAL X50 Preservative contains the new chemical 2-methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI) as its active ingredient at approximately 5% in water. PROMEXAL X50 is a preservative for aqueous compositions such as oil in water emulsions, lattices, emulsion paints, water based adhesives, casein and rosin dispersions, textile spin-finish solutions, aqueous slurries, titanium dioxide slurries and tape joint compounds.

II. Examination of the toxicology data base for MTI (active ingredient) and PROMEXAL X50 Preservative .

The applicant has submitted toxicology data (MRID Nos. 432250-02 and 431387-14 through 431387-34) for the technical grade of the active ingredient (MTI) and the formulation PROMEXAL X50 Preservative.

These studies have been evaluated and the Data Evaluation Reports (DERs) are attached. The conclusions of the evaluations are summarized below in Tables 1 (acutes) and 2 (non-acutes) and appear in detail in Section III of this memorandum.

As summarized in Table 1 for acute toxicity data, MTI technical is a skin sensitizer and has been placed in Toxicity Category I for dermal toxicity and for

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eye irritation. Additionally, MTI technical has been placed in Toxicity Categories II for oral toxicity and IV for skin irritation. PROMEXAL X50 is also a skin sensitizer and has been placed in Toxicity Category I for eye irritation. Additionally, PROMEXAL X50 has been placed in Toxicity Categories II for skin irritation and III for oral and dermal toxicity.

Concerning acute inhalation toxicity, no acute inhalation toxicity data were available for examination. Due to the possible use of PROMEXAL X50 in emulsion paints (which may result in an aerosol/particulate exposure), acute inhalation toxicity studies (for the EP and the TGAI) are required. Acute inhalation studies (for the EP and the TGAI) are required if the product consists of, or under conditions of use will result in an inhalable material (e.g. gas, volatile substances, or aerosol/particulate).

Concerning mutagenicity testing, MTI technical was found to be positive with and without metabolic activation in the cultured human lymphocytes assay. Additionally, MTI was weakly positive in the mouse micronucleus assay at a dose level up to 136 mg/kg. The HED RfD/Peer Review Committee (meeting of May 5, 1995) concluded that the submitted mutagenicity studies (MRID Nos. 431387-28, 431387-29 and 431387-30) satisfy the minimum mutagenicity testing in the three categories of mutagenicity testing (pre-1991 Guidelines). It was noted however, that those tests were done after 1991 and should have followed the current Guidelines. According to the current Guidelines the submitted battery is missing an in vitro mammalian gene mutation assay. However, based on the positive results in the two above cytogenetic assays, a follow-up test is needed and the dominant lethal assay is the test of choice. Thus, a dominant lethal assay is required in addition to the tests already submitted.

A carcinogenicity study in two species is conditionally required for a non-food pesticide [40 CFR §158.340 (Guideline 83-2)] that is mutagenic as demonstrated by in vitro or in vivo testing. Such studies are not required at this time for MTI based on the weight of the available evidence.

III. Additional Non-Guideline studies

In addition to the studies submitted to fulfill registration requirements, the Registrant has submitted 2 other studies containing supplementary information on the chemical.

1. Assessment of the Relative Skin Sensitizing Potency of 3 Biocides Using the Murine Local Lymph Node Assay. P.A. Botham, J, Hilton, C.D. Evans, D. Lees, T.J. Hall. Contact Dermatitis 25: 172-177 (1991). In this published article the relative skin-sensitizing potency of the 3 following biocides was studied:

- o 5-Chloro-2-methyl-4-isothiazolin-3-one (the major a.i. in Kathon CG)
- o 1,2-benzisothiazolin-3-one (BIT)
- o 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI, subject of this memo)

Experimental: In brief, groups of 4 mice/dose level were treated (daily for 3 days) in the dorsum of both ears with dilutions of the test material in DMF. Controls were treated with vehicle alone. Five days after the initial dosing, all mice were injected with tritiated thymidine in buffered saline and sacrificed

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5 hours later. The draining auricular nodes were removed and single-cell suspensions of lymph node cells were prepared. Incorporation of tritiated thymidine into the lymph node cells was determined by LSC. The authors indicated that a 3-fold or greater increase in the mean incorporation of tritiated thymidine/node vs. untreated controls is a reliable indicator of sensitization potential.

Results: The results for the various concentrations of the 3 biocides are shown in Attachment 1. As summarized by the authors:

- o CMIT (the major active ingredient in Kathon) induced lymphocyte proliferation (28-fold increase) at concentrations of 0.1 to 0.3%
- o MTI (subject of this memo) was unable to stimulate a response at levels below 3%.
- o BIT had no effect below 10%
- o 10% MTI showed a 9-fold increase in lymphocyte proliferation.
- o 50% BIT showed a 5-fold increase in lymphocyte proliferation.
- o These studies suggest that CMIT is a significantly more potent skin sensitizer than either BIT or MTI.

Discussion: As summarized above, these studies suggest that CMIT (the main ingredient of Kathon) is a significantly more potent skin sensitizer than either BIT or MTI. This study provides supplementary information on MTI. The dermal sensitization study in guinea pigs with MRID No. 431387-22, has already fulfilled the Guideline 81-6 requirement for MTI technical.

2. MTI: Assessment of Oral vs. Dermal Toxicity. P.H. Rose. January 10, 1994. Unpublished Study. Zeneca Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK. Laboratory Project ID CTL/PHR/10194. MRID 431387-33.

In this brief document (Attachment 2) the author reviews the toxicity of MTI in rats. By comparing the acute dermal and oral toxicities of MTI, the author concluded that MTI is at least 20 times more toxic systemically by the oral route than by the dermal route. Based on the postulated ratio of 1:20 for dermal to oral acute systemic toxicities and the oral subchronic NOEL and LOEL values of 4.1-4.6 and 21-23 mg/kg/day, respectively, in rats, the authors postulated dermal subchronic NOEL and LOEL values of 82-92 and 420-450 mg/kg/day, respectively.

Based on the slight to moderate skin irritation observed at 50 mg/kg during acute toxicity testing, the author concluded that at or above the predicted dermal NOEL values for systemic toxicity there would be unacceptable levels of skin irritation upon repeated dermal exposure to MTI. This would preclude an assessment of the systemic toxicity of MTI by the dermal route.

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Table 1. Results of acute toxicity testing with MTI Technical and PROMEXAL X50 Preservative.

Guideline #	Test Type	Species	MRID	LD_{50} (mg/kg)	Remarks	Toxicity Category	CORE Classification
				MTI (a.i.)			
81-1	Acute Oral	Rat	431387-18	224 (M) & 168 (F)	Hemorrhagic stomach at 500 mg/kg	II	Minimum
81-2	Acute Dermal	Rat	431387-19	Not determined	Animals were sacrificed due to extreme skin irritation	I	Minimum
81-3	Acute Inhalation	-	-	-	No data available for review[1]	-	-
81-4	Eye Irritation	Rabbit	431387-20	-	Animals sacrificed due to severity of eye irritation	I	Minimum
81-5	Skin Irritation	Rabbit	431387-21	-	No erythema or edema (Grades = 0) seen at 72 hours	IV	Minimum
81-6	Dermal Sensitization	Guinea pig	431387-22	-	Extreme sensitizer by this test.	-	Minimum
				PROMEXAL X50			
81-1	Acute Oral	Rat	431387-14	2359 (M) & 1831 (F)	Local irritant to the stomach	III	Minimum
81-2	Acute Dermal	Rat	431387-15	>2000 (M&F)	No signs of systemic toxicity	III	Minimum
81-3	Acute Inhalation	-	-	-	No data available for review[1]	-	-
81-4	Eye Irritation	Rabbit	431387-16	-	In vitro test indicates potential for severe ocular irritation in vivo	I	Acceptable[2]
81-5	Skin Irritation	Rabbit	432250-02	-	Severe skin irritation for more than 72 hours	II	Minimum
81-6	Dermal Sensitization	Guinea pig	431387-17	-	A moderate sensitizer under the conditions of testing	-	Minimum

[1] A study required to meet this guideline.
[2] It is noted that this test is not a standard Guideline §81-5 study. This study is accepted as fulfilling Guideline §81-5 because the results of the test are clearly indicative of a severe irritation potential and allow for the conservative placement of the test material in Toxicology Category I.

Table 2. Results of testing (other than acute toxicity) with MTI Technical and PROMEXAL X50 Preservative.

Guideline #	Test type	Species	MRID	Systemic (M/F) or Maternal		Develop. or Reproduct.		Remarks	CORE Classification
				LOEL	NOEL	LOEL	NOEL		
				mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day		
82-1	90-D feeding	Rat	431387-24	20.7/23.2	4.1/4.6	-	-	LOEL based on decreases in food efficiency and body weight gains	Minimum
"	"	Dog	431387-26	Not Detnd.	31.5/33.4	-	-	No apparent treatment-related effects	Minimum
83-3	Develop. (Oral)	Rat	431387-27	40 (HDT)	15	Not Detnd.	40 (HDT)	No evidence of developmental toxicity	Minimum
84-2	Ames/Salmonella	-	431387-28	-	-	-	-	Negative (+ metabolic activation)	Acceptable
"	In vitro human lymphocytes/aberr.	-	431387-30	-	-	-	-	Positive (+ metabolic activation)	Acceptable
"	Unscheduled DNA synthesis	Rat	431387-31	-	-	-	-	Negative at doses of 76, 117 or 180 mg/kg, and sacrifices at 4 and 12 hours postdosing	Acceptable
"	Micronucleus	Mouse	431387-29	-	-	-	-	Weakly positive at a dose level up to 136 mg/kg	Acceptable

IV. Summaries of DERs for the submitted toxicology studies with MTI (Technical) and PROMEXAL X50 Preservative

1. Acute Oral Toxicity in Rats (Guideline §81-1) - MRID 431387-18

Groups of young adult SPF Wistar-derived (Alpk : APfSD) albino rats (5 /sex/dose) were given a single oral dose of MTI (95% a.i) in deionized water at doses of 50, 100 or 500 mg MTI/kg b.w. Necropsy revealed blotchy livers and hemorrhagic stomach and intestines in all animals dosed with 500 mg/kg; these effects were not observed at lower doses.

LD_{50}: ♂: 224 mg/kg b.w. ♀: 168 mg/kg b.w.
Toxicity Category: II
Classification: Minimum.

2. Acute Oral Toxicity in Rats (Guideline §81-1) - MRID 431387-14

Groups of young adult SPF Wistar derived albino rats, (Alpk : APfSD), 5 rats/sex/dose, were given a single oral dose of PROMEXAL X50 (4.85% a.i.) in deionized water at doses of 1000, 2000 or 3500 mg PROMEXAL X50/kg b.w. and observed for 15 days. Macroscopic findings were consistent with the formulation acting as a local irritant to the stomach.

LD_{50}: ♂: 2359 mg/kg b.w. ♀: 1831 mg/kg b.w.
Toxicity Category: III
Classification: Minimum.

3. Acute Dermal Toxicity in Rats (Guideline §81-2) - MRID 431387-19

Groups (Wistar-derived, Alpk : APfSD) of rats (7-9 weeks old, 2 males or 2 females/dose) were administered dermally MTI Technical as a paste at doses of 50, 200 or 400 mg/kg b.w. and as an aqueous formulation at doses of 200, 400 or 1000 mg/kg b.w. to a shaved area of the dorsal trunk and covered with a 24 cm^2 patch for 24 hours. With the paste at 200 mg/kg, both animals had large areas of skin necrosis and one rat was sacrificed on day 5 due to skin irritation; at 400 mg/kg both animals were sacrificed on day 3 due to skin irritation. With the aqueous formulation at 1000 mg/kg, both animals were sacrificed on day 5 due to skin irritation.

LD_{50}: Not determined. Rats sacrificed due to extreme skin irritation.
Toxicity Category: I
Classification: Minimum.

4. Acute Dermal Toxicity in Rats (Guideline §81-2) - MRID 431387-15

Groups (SPF Wistar-derived, Alpk : APfSD) of young albino rats (5/sex) were administered dermally PROMEXAL X50 (4.85% w/w MTI) at a dose of 2000 mg/kg b.w. (approximately 97 mg MTI/kg b.w.) to a shaved area of the dorsal trunk

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and covered with a 24 cm^2 patch for 24 hours. There were no signs of systemic toxicity. Slight to moderate erythema and edema were observed during the first 5-6 days of the observation period; slight desquamation was observed up to days 12-13 of the observation period. There were no male deaths and at most 1 female death.

LD_{50} : > 2000 mg/kg for both sexes.
Toxicity Category: III
Classification: Minimum.

5. Primary Eye Irritation in Rabbits (Guideline §81-4) - MRID 431387-20

One male New Zealand White rabbit from Mellor Rabbits, Chadderton Heights, Chadderton, Nr Oldham, Greater Manchester, UK. was treated in the conjunctival sac with 50 mg of MTI Technical. Approximately one hour after application, the rabbit was seen to be in distress. Signs of ocular irritation included severe redness and chemosis of the conjunctiva, a slight discharge and moderate erythema of the eyelids. The animal was sacrificed 1.5 hours after application.

Toxicity Category: I
Classification: Minimum.

6. Primary Eye Irritation in Rabbits (Guideline §81-4) - MRID 431387-16

In an in vitro test, two isolated eyes from New Zealand White rabbits (source unspecified) were exposed for 10 seconds to 0.1 ml undiluted PROMEXAL X50. Following rinsing of the eyes with saline, corneal swelling was determined at 0.5, 1, 2, 3, 4, and 5 hours after dosing. Swelling increased with time, from 10.5% at 0.5 hours to 86.8% at 5 hours. Based on the large corneal swelling (> 15%), it was concluded that PROMEXAL W50 is likely to be a severe ocular irritant in vivo.

Toxicity Category: I
Classification: Acceptable.

7. Primary Dermal Irritation in Rabbits (Guideline §81-5) - MRID 431387-21

One sq. inch areas of intact skin of New Zealand White rabbits (3 males) from Mellor Rabbits, Chaddertton Heights, Greater Manchester, UK were exposed to 500 mg of MTI Technical moistened with 0.1 ml deionized water for 4 hours. Very slight to well defined erythema (Grades 1-2) and well defined edema (Grade 2) were seen in all animals at 30-60 minutes after patch removal. No erythema or edema (Grades = 0) were seen at 72 hours after dosing.

Toxicity Category: IV
Classification: Minimum.

8. Primary Dermal Irritation in Rabbits (Guideline §81-5) - MRID 432250-02

One sq. inch areas of intact skin of New Zealand White rabbits (6 males) from the Conventional Animal Breeding Unit. Alderley Park, Macclesfield, Cheshire, (UK) were exposed to 0.5 ml of undiluted PROMEXAL X50 (4.85% w/w MTI) for 4 hours. Severe erythema was observed in 5/6 rabbits, together with severe edema in all rabbits at 30-60 minutes after dosing. On day 3, 1/6 rabbits had severe erythema and 4/6 rabbits had moderate to severe erythema. On day 3, 3/6 rabbits had severe edema and 2/6 had moderate to severe edema. One rabbit still had severe erythema and another one still had severe edema on day 7. Erythema persisted in two rabbits up to day 21 after treatment.

Toxicity Category: II
Classification: Minimum.

9. Dermal Sensitization in Guinea Pigs (Guideline §81-6) - MRID 431387-22

In a Buehler test, 20 albino Porcellus Dunkin Hartley female guinea pigs from Harlan Porcellus, Firgrove Farm, Heathfield, Sussex, UK. were induced dermally with a 3% (w/v) preparation of the test material in deionized water. Induction was done in a total of 3 six-hour exposures (the interval between exposures was 7 days)to the test material. Two weeks after the final induction, the test animals were challenged dermally with 1% (w/v) and 0.3% (w/v) preparations of the test material in deionized water. Challenge with the 1% (w/v) preparation produced a net percentage response of 70%, which falls in the strong sensitizer category. Challenge with the 0.3% (w/v) preparation produced a net percentage response of 90%, which falls in the extreme sensitizer category. Technical MTI was found to be an extreme sensitizer under the conditions of this test.

Classification: Minimum.

10. Dermal Sensitization in Guinea Pigs (Guideline §81-6) - MRID 431387-17

In a Buehler test, 20 albino Alpk:Dunkin Hartley female guinea pigs from the Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK were induced dermally with undiluted PROMEXAL X50. Induction was done in a total of 3 six-hour exposures (the interval between exposures was 7 days)to the test material. Two weeks after the final induction, the test animals were challenged dermally with 30%, 10%, 3% and 1% w/v dilutions of the test material in deionized water. Challenge with the 30% and 10% (w/v) dilutions produced net percentage responses of 63 and 44%, respectively, which fall in the moderate sensitizer category. Challenge with the 3% (w/v) dilution produced a net percentage response of 5%, which falls in the weak sensitizer category; the 1% solution did not elicit any sensitization response. PROMEXAL W50 was found to be a moderate sensitizer under the conditions of this test.

Classification: Minimum.

11. 90-Day feeding - Rats. (Guideline §82-1) - MRID 431387-24

MTI was administered to Alpk:APfSD (Wistar derived) rats of both sexes for a period of 90 days in the diet at dose levels of 0, 50, 250, or 1000 ppm, corresponding to mean MTI intake of 0, 4.1, 20.7 or 83 mg/kg/day (in males) and 0, 4.6, 23.2, or 93.0 mg/kg/day (in females). Statistically significantly decreased body weights were observed at 1000 ppm in males throughout the treatment period (e.g. to 84.8% of controls at 14 weeks). Mean body weight gains at 250 and 1000 ppm in males were reduced to 95.2 and 79.3% of controls for weeks 1-14. Mean food efficiency values for high dose males were statistically significantly decreased for weeks 1-13 (to 89.7% of controls). Statistically significantly decreased body weights were observed at 250 and 1000 ppm in females throughout the treatment period (e.g. to 94.5 and 87.8% of controls at 14 weeks for 250 and 1000 ppm, respectively). Likewise, mean body weight gains decreased in dose-related fashion to 89.1% and 68.7% of controls at 250 and 1000 ppm, respectively, at 1-5 weeks and to 92.6% and 76.7% of controls, at 250 and 1000 ppm, respectively, for weeks 1-14. Mean food efficiency values for weeks 1-4 decreased with increasing dose, reaching statistical significance at 250 and 1000 ppm, at 91.3 and 76.5% of controls, respectively. There were no apparent toxicologically significant findings in clinical chemistry, hematology, or macroscopic and microscopic pathology.

LOEL : 250 ppm (♂: 20.7 mg/kg/day; ♀: 23.2 mg/kg/day), based on dose-related and statistically significant, decreases in food efficiency for females at 250 and 1000 ppm for weeks 1-4), coupled to dose-related decreases in body weight gain at 250 and 1000 ppm for weeks 1-4).
NOEL : 50 ppm (♂: 4.1 mg/kg/day; ♀: 4.6 mg/kg/day).

Classification: Minimum.

12. 90-Day feeding - Dogs. (Guideline §82-1) - MRID 431387-26

MTI was administered to beagle dogs of both sexes for a period of 90 days in the diet at dose levels of 0, 100, 300, or 1000 ppm, corresponding to mean MTI intake of 0, 3.1, 9.3 or 31.5 mg/kg/day (in males) and 0, 3.2, 10.1, or 33.4 mg/kg/day (in females). There were no effects on body weights or body weight gains for both sexes. These effects are in contrast with those observed in the pilot study [MRID 431387-25]: body weight gains in females at 928 and 464 ppm in the pilot study were 36.4% and 54.5% of those of controls, respectively. Changes in hematology and clinical chemistry parameters did not follow a clear dose-related trend and appeared to be incidental to the treatment. No clear-cut, dose-related effects were observed upon macroscopic examination of the dogs. Although clear macroscopic signs of irritation of the tongue were seen in 1392 and 928 ppm females in the pilot study, no signs of tongue irritation were seen in this study. There were no apparent treatment-related histological findings.

LOEL: Not defined in this study due to the absence of clear-cut, dose-related effects.

NOEL: 1000 ppm, the highest dose tested (♂: 31.5 mg/kg/day; ♀: 33.4 mg/kg/day).

Classification: Minimum.

13. Developmental Toxicity - Oral- Rats. [Guideline §83-3(a)] - MRID Nos. 426849-25 (Main study) and 426849-30 (Pilot).

Mated female rats (groups of 24/dose) were gavaged during gd 7-16 (inclusive) at dose levels of 0, 5, 15, and 40 mg/kg/day. Maternal toxicity was established for the HDT by: (1) the observation of two deaths observed on gds 12 and 13 (1 in extremis, 1 found dead), (2) clinical signs of toxicity in 4 dams (including the two which died) related to the respiratory system (abnormal noise, labored breathing, gasping, reduced breathing rate), (3) statistically significant depressions in mean body weights (gds 8-16 inclusive) of 4-6.5% which remained decreased following cessation of dosing, (4) a 62% decrement in mean body weight gain during the dosing period, and (5) statistically significant depressions in mean food consumption on gds 7-10, 10-13 and 13-16. There were no apparent dose-related effects upon mean implantations/dam, live fetuses/dam, resorptions/dam (early, late), postimplantation losses, the sex ratios, mean fetal weights or external/visceral/skeletal anomalies.

Maternal LOEL : 40 mg/kg/day, the highest dose tested, based on increased mortality and clinical signs (respiratory system), depressed mean body weight and weight gain and decreased food consumption.
Maternal NOEL : 15 mg/kg/day.

Developmental LOEL : Not defined in this study, there was no evidence of developmental toxicity.
Developmental NOEL : 40 mg/kg/day (the highest dose tested).

Classification: Minimum.

14. Gene Mutation Assay. Reverse Mutation Test in Salmonella and E.Coli (Guideline §84-2) - MRID No. 431387-28.

In an initial microbial/mammalian microsome plate incorporation assay Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, and Escherichia coli strain WP2uvrA pKM101 were exposed to 0.32, 1.6, 8.0, 40, 100, or 200 μg/plate of MTI in the presence or absence of S9 activation derived from Arochlor 1254 induced rat liver. For the repeat trial, nonactivated levels of 0.16, 0.8, 4.0, 20, 50, or 100 μg/plate were assayed with the Salmonella strains and 0.064, 0.32, 1.6, 8.0, 20, or 50 μg/plate were assayed with E. Coli. Doses evaluated under S9-activated conditions were similar to those used in the initial trial. Dimethyl sulfoxide was used as the solvent. Although slight but significant and dose-related increases in revertant colonies of strain TA 1535 were seen in the S9-activated phase of the initial assay, the effect was not reproducible and, therefore, did not provide sufficient evidence of a mutagenic response. MTI

was cytotoxic (≥100 μg/plate -S9; ≥200 μg/plate +S9) in all strains but failed to induce a mutagenic effect. All strains responded to the mutagenic action of the appropriate positive control.

Classification: Acceptable

15. In vitro cytogenetic assay with cultured human lymphocytes (Guideline §84-2) - MRID No. 431387-30.

In an in vitro cytogenetic assay, cultured human lymphocytes, obtained from one male and one female donor were exposed to MTI doses of 2, 10, or 20 μg/ml -S9 (male donor); 1, 5, or 10 μg/ml -S9 (female donor) or 2, 10, or 20 μg/ml +S9 (both donors). The test material was delivered to the test system in dimethyl sulfoxide, and the S9 was derived from Aroclor 1254 induced rat liver. Significant ($p<0.01$) clastogenic effects were seen at 10 and 20 μg/ml without S9 activation and at 20 μg/ml with S9 activation in lymphocytes derived from the male donor and at 10 and 20 μg/ml +S9 in cells derived from the female donor. Higher levels with or without S9 activation were severely cytotoxic. The test is positive in the presence and absence of metabolic activation at concentrations as low as 10 μg/ml. The response is more pronounced in the presence of metabolic activation.

Classification: Acceptable

16. Mouse micronucleus assay (Guideline §84-2) - MRID No. 431387-29

In a mouse micronucleus assay (MRID No. 431387-29), groups of five male and five female C57BL/6 mice received single oral gavage administrations of 85 or 136 mg/kg (males) or 103 or 164 mg/kg (females) MTI delivered in physiological saline. At 24, 48 or 72 hours post- exposure, high dose animals were sacrificed and bone marrow cells were examined for micronucleated polychromatic erythrocytes (MPEs). Bone marrow cells were harvested from animals in the low-dose groups only at 24 hours after treatment. Unscheduled deaths occurred in five high-dose males and four high-dose females; dead animals were replaced with mice from a secondary group. A significant ($p<0.05$) decrease in the ratio of normochromatic to polychromatic erythrocytes (PCEs), indicating cytotoxic effects on the target organ, was seen in the high-dose groups (both sexes) at the 24-hour sacrifice. A significant ($p<0.01$) increase in mean MPEs for males administered 136 mg/kg at 24 hours prompted the study investigators to evaluate an additional 2000 PCEs/male from the 24- and 48-hour sacrifice groups for the high dose and from the 24-hour sacrifice group for the low dose. Based on this expanded analysis, the data showed nonsignificant increases in MPEs for high-dose males at both sacrifice times. A similar response was present in high-dose females at 48 hours. The study authors attributed the increase in males (48 hr.) to a single animal having high MPE counts (14/1000--initial count; 13/1000 and 12/1000 --subsequent extended counts). However, increases in the number of micronuclei were seen in individual animals as follows: 2 high-dose males (24 hrs)--4.3 or 4.0 MPEs/1000 PCEs vs. 2.1 MPEs/1000 PCEs (controls)

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2 high-dose males (48 hrs)--3.3 or 13.0 MPEs/1000 PCEs vs. 1.7 MPEs/1000 PCEs (control)
1 low-dose male (24 hrs)--4.7 MPEs/PCEs vs. 2.1 MPEs/1000 PCEs (controls)
1 high-dose female (48 hrs)--10 MPEs/1000 PCEs vs. 1.8 MPEs/1000 PCEs.

Although the statistical significance of the results was lost owing to the wide variability in the data, the increased sample size (3000 PCEs/males at all doses and sacrifice times) increases our confidence in the test system's ability to predict a doubling over background as a true biological effect. It is also of note that MTI induced an unambiguous clastogenic response in cultured human lymphocytes (see MRID No.431387-30). It is concluded, therefore, that the results in this mouse micronucleus assay are consistent with a weak clastogenic and/or aneugenic response.

Classification: Acceptable

17. In vivo/in vitro unscheduled DNA synthesis assay in primary rat hepatocytes following in vivo dosage. Guideline §84-2. MRID 431387-31.

In an in vivo/in vitro unscheduled DNA synthesis (UDS) study (MRID No. 431387-31), groups of five male rats were administered single oral gavage doses of 76, 117, or 180 mg/kg MTI prepared in deionized water. The high dose was estimated to be 80% of the male rat oral LD_{50}. Animals were sacrificed at 4 and 12 hours posttreatment and recovered hepatocytes were scored for UDS Two independent trials were performed.

Clinical signs of toxicity noted immediately after dosing included salivation, difficulty in breathing and staining and fluid around the nose. Treatment with MTI produced no evidence of cytotoxicity for the target cells. There was also no evidence of a genotoxic response at any dose or sacrifice time.

Classification: Acceptable.

Attachment 1

From: Assessment of the Relative Skin Sensitizing Potency of 3 Biocides Using the Murine Local Lymph Node Assay.
P.A. Botham et al. Contact Dermatitis 25: 172-177 (1991).

the EEC. require the use of a guinea pig assay, such as the maximization test (12) or the occluded patch test (13) for the notification of new biocides (14). These assays assess the absolute potential of a substance to cause allergic contact dermatitis. There is no requirement to perform dose-response studies of the type used to determine the minimum sensitizing dose of Kathon® CG (3) and, in any case, these studies are difficult to perform and require large numbers of animals. There is therefore little information on the relative sensitization dose-response relationships for biocides and thus on the relative sensitization potencies of these materials.

We have used the murine local lymph node assay (15) to compare the potency of 3 biocides, the major active ingredient in Kathon® CG, BIT and MTI (2-methyl-4,5-trimethylene-4-isothiazolin-3-one). Potency was ranked according to the lowest dose of material which, following epicutaneous exposure of mice, induced a significant proliferation of T lymphocytes in the draining lymph nodes.

Material and Methods

Mice

Young adult (6–8 week) CBA/Ca strain mice were used throughout.

Chemicals

5-chloro-2-methyl-4-isothiazolin-3-one, 1,2-benzisothiazolin-3-one and 2-methyl-4,5-trimethylene-4-isothiazolin-3-one were obtained pure (>97%, 100% and >97%, respectively). Each test sample was dissolved or suspended in dimethyl formamide (DMF) to give a range of concentrations to be applied to the mice.

Measurement of lymphocyte proliferation

Groups of 4 mice were dosed with a single concentration of a test substance in DMF; a group of control animals received the vehicle alone. The preparations (25 μl) were administered to the dorsum of both ears using a micropipette. The treatment was performed daily for 3 consecutive days.

5 days following the initial dosing, all mice were injected i.v. via the tail vein with 250 μl of phosphate-buffered saline (PBS), containing 20 μCi of ^{3}H-methyl thymidine (specific activity 2 Ci/mmol; Amersham International, Amersham, UK). 5 h later, the mice were sacrificed and the draining auricular lymph nodes were removed and pooled for each experimental group.

A single cell suspension of lymph node cells was prepared by mechanical disaggregation through a 200-mesh stainless steel gauze. The cell suspensions were washed in PBS and precipitated in 5% (w/v) trichloroacetic acid (TCA). Following centrifugation, the samples were resuspended in TCA and transferred into 10 ml of scintillation fluid (Optiphase MP; LKB). Incorporation of ^{3}HTdR was measured by β-scintillation counting and expressed as mean cpm/node for each group. The activity of each test group was divided by the activity of the control (vehicle only) group to give a test/control ratio (increment) for each concentration of each chemical tested.

Experience with the assay has shown that a 3-fold or greater increase in the mean incorporation of ^{3}HTdR/node is a reliable indicator of sensitization potential (15).

Results

In a first series of experiments, groups of mice were exposed to a range of concentrations (3, 10, 30 and 50% w/v) of 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), BIT and MTI in DMF or to DMF alone. The results are shown as experiment no. 1 in Tables 1–3. CMIT was extremely toxic at these dose levels: animals which received the 10, 30 or 50% preparations all died or were killed *in extremis* due to the systemic toxicity of the material. However, significant lymphocyte proliferation (i.e., a 3-fold or greater increase in the mean incorporation of ^{3}HTdR in the nodes of test animals compared to vehicle-treated controls) was seen in

Table 1. Induction of lymphocyte proliferation in draining lymph nodes following exposure to 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT – the major active ingredient in Kathon® CG)

Test chemical concentration (% w/v)	^{3}HTdr incorporation (mean cpm/node × 10^{-3})	Increment
Experiment no. 1		
0	0.98	–
3	7.02	7.16
10,30,50	toxic	–
Experiment no. 2		
0	0.65	–
3	10.86	16.71
1	11.14	17.14
0.3	18.25	28.08
Experiment no. 3		
0	1.26	–
1	27.92	22.16
0.3	32.35	25.67
0.1	34.94	27.73
0.03	15.51	12.31
0.01	4.50	3.57
0.003	1.12	0.89
0.001	1.29	1.02

mice treated with 3% CMIT (Table 1), in animals which had received 50% BIT (Table 2) and in all 4 groups of animals which had been treated with MTI (Table 3).

Table 2. Induction of lymphocyte proliferation in draining lymph nodes following exposure to 1,2-benzisothiazolin-3-one (BIT)

Test chemical concentration (% w/v)	^{3}HTdr incorporation (mean cpm node × 10^{-3})	Increment
Experiment no. 1		
0	1.00	–
50	4.53	4.53
30	2.79	2.79
10	1.22	1.22
3	1.56	1.56
Experiment no. 2		
0	1.26	–
50	6.26	4.97
30	5.61	4.45
10	4.84	3.84
3	3.43	2.72

Table 3. Induction of lymphocyte proliferation in draining lymph nodes following exposure to 2-methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)

Test chemical concentration (% w/v)	^{3}HTdr incorporation (mean cpm/node × 10^{-3})	Increment
Experiment no. 1		
0	0.57	–
50	2.78	4.88
30	3.99	7.00
10	5.12	8.98
3	2.59	4.54
Experiment no. 2		
0	1.26	–
10	7.79	6.18
3	4.49	3.56
1	3.26	2.59
0.3	1.57	1.25

As these initial experiments did not establish, at least for CMIT and MTI, the lowest dose level which was capable of inducing significant proliferative responses, a second series of experiments was conducted using lower dose levels. The results are shown as experiment nos. 2 and 3 in Tables 1–3. CMIT induced lymphocyte proliferation at dose levels as low as 0.01%, with maximal proliferation (28-fold) occurring at 0.1–0.3%. In contrast, MTI was unable to stimulate a response at levels below 3% and BIT had no effect below 10%; proliferative activity was highest in animals treated with 10% MTI and 50% BIT, but was only 9-fold and 5-fold, respectively. Representative dose-response curves are shown in Fig. 1.

Discussion

A number of methods, mostly in the guinea pig, have been developed over the last 30 years to assess the skin sensitization potential of chemicals (16). Practical experience has shown that these methods can successfully be used in preventing allergic contact dermatitis. However, they provide an indication only of the potential of a substance to cause skin sensitization. In addition, different results may arise

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Attachment 2
From: MTI: Assessment of Oral vs. Dermal Toxicity. P.H. Rose.
MRID 431387-33

EPA Reg. NO. 10182-385

Page_____ is not included in this copy.

Pages 18 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

011613

Reviewed by: Alberto Protzel, Ph.D. 5/31/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: Steven L. Malish, Ph.D. 5/31/95
Section III, Tox. Branch II(7509C)

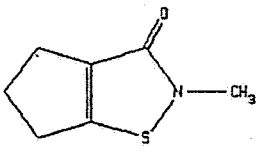
DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity in rats;
EPA Guideline 81-1

EPA IDENTIFICATION: EPA MRID No. 431387-14
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: PROMEXAL W50 (4.85% w/w formulation of MTI)

SYNONYMS/STRUCTURE: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)



STUDY NUMBER: Report No. CTL/P/3737; Study No. AR5407

SPONSOR: ICI Americas Inc. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: PROMEXAL W50: Acute Oral Toxicity to the Rat.

AUTHOR: P. Robinson

REPORT ISSUED: July 31, 1992 (Study Director signature dated July 6, 1992).

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID No. 431387-14), groups of young adult SPF Wistar derived albino rats, (Alpk : APfSD), 5 rats/sex/dose, were given a single oral dose of PROMEXAL W50 (4.85% a.i.) in deionized water at doses of 1000, 2000 or 3500 mg PROMEXAL W50/kg b.w. and observed for 15 days.

LD_{50} : Males = 2359 mg/kg b.w.
Females = 1831 mg/kg b.w.

Macroscopic findings were consistent with the formulation acting as a local irritant to the stomach.

The study is classified as Acceptable with a Toxicity Category III and satisfies the requirement, § 81-1 for an acute oral toxicity study in rats.

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MATERIALS:

1. Test compound: PROMEXAL X50 (sample reference NBY 1919/24); Description: pale yellow liquid; Lot. No.: Unspecified (CTL reference number was Y06823/008); Purity: 4.85% w/w MTI [As stated in a certificate of analysis with reference No. NBY1457/48. The certificate of analysis was not included with the report]. Preparations were prepared in deionized water.

2. Test animals: Species: rat, Strain: Specific pathogen free (SPF) Wistar derived albino rats, (Alpk : APfSD); Age: Young adult; Weight (Day -1): 298-350 g (males) and 220-271 g (females); Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.

3. Animal husbandry: The rats were housed in suspended cages; a maximum of five rats in each cage, and the sexes were kept separately. The animals were allowed free access to food (Porton Combined Diet) and given unlimited access to water via an automatic system. The rats were acclimatized a minimum of 6 days.

METHODS:

Dose levels for the main study were selected on the basis of preliminary range finding study.

Rats were fasted for up to 24 hours before dosing. Test material as a preparation in deionized water was administered orally (by gavage) to 5 rats/sex/dose at a rate of 10 ml/kg to give doses of 1000, 2000 or 3500 mg PROMEXAL W50/kg b.w. Animals were observed for signs of toxicity once within 2 hours after dosing and again between 4 and 7 hours after dosing and once daily (or more often if there were signs of toxicity) up to day 15. Rats were weighed on days -1, 1 (day of dosing), 3, 4, 8 and 15. Animals in extremis and animals surviving at the end of the study were sacrificed, necropsied and examined for macroscopic abnormalities.

The acute oral median lethal dose was calculated separately for males and females the mortality data by logistic regression using nominal dose values. Confidence limits were calculated using a likelihood ratio interval.

RESULTS:

Cumulative mortality (including animals killed in extremis) is presented in Table 1. Treatment-related clinical signs of toxicity included:

- Males: There were no deaths or signs of systemic toxicity at 1000 mg/kg. At 2000 mg/kg, signs included salivation, sides pinched, subdued appearance. At 3500 mg/kg, the sole survivor had piloerection, sides pinched in, staining around the nose and mouth and upward curvature of the spine with recovery evident by day 5.

- Females: There were no deaths, and except for one rat, there were no signs of systemic toxicity at 1000 mg/kg. Signs of slight to moderate toxicity (including breathing irregularities, decreased activity and tip toe gait)

were presented at 2000 mg/kg, with recovery evident by day 7.

Although all animals lost weight initially due to the pre-dose fast, all survivors had exceeded their initial weights at termination.

Macroscopic findings at necropsy were consistent with the formulation acting as a local irritant to the stomach. These findings included red areas in the glandular region of the stomach and distension of the stomach associated with gas production or increased fluidity of the contents. A diverticulum in the stomach of a 1000 mg/kg female resulted from acute necrosis, inflammation, and a granulation response affecting the stomach wall; the lesion may have been exacerbated by catheter damage for more pronounced lesions were not seen at higher dose levels.

Table 1. Cumulative mortality in rats after a single oral dose of PROMEXAL W50[1].

Dose mg/kg	Males [deaths/dosed]	Females [deaths/dosed]
1000	0/5	0/5
2000[2]	2/5	3/5
3500[3]	4/5	5/5
LD_{50}(95% CL)[4] =	2359 (1577-4034) mg/kg	1831(1386-2420) mg/kg

[1] From p. 84 of the Study Report (MRID 431387-14).
[2] Two males and 3 females were killed in extremis or were found dead due to toxicity between days 1 and 9.
[3] All females and 4 males were killed in extremis or were found dead due to toxicity on the day of dosing.
[4] 95% confidence limits.

REGULATORY COMPLIANCE: Signed and dated quality assurance, GLP compliance and no confidentiality claim statements were present.

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011613

Reviewed by: Alberto Protzel, Ph.D. 5/31/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: Steven L. Malish, Ph.D. 5/31/95
Section III, Tox. Branch II(7509C)

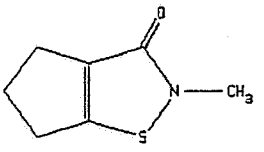
DATA EVALUATION REPORT

STUDY TYPE: Acute oral toxicity in rats;
EPA Guideline 81-1

EPA IDENTIFICATION: EPA MRID No. 431387-18
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: Report No. CTL/P/2791

SPONSOR: Zeneca Inc. Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one: Acute Oral Toxicity to the Rat.

AUTHOR: J.C. McCall

REPORT ISSUED: January 18, (Study Director signature dated January 11, 1990).

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID No. 431387-18), groups of young adult SPF Wistar-derived (Alpk : APfSD) albino rats (5 /sex/dose) were given a single oral dose of MTI (95% a.i) in deionized water at doses of 50, 100 or 500 mg MTI/kg/b.w.

LD_{50} : Males = 224 mg/kg b.w.
Females = 168 mg/kg b.w.

Necropsy revealed blotchy livers and hemorrhagic stomach and intestines in all animals dosed with 500 mg/kg; these effects were not observed at lower doses.

The study is classified as Acceptable with a Toxicity Category II and satisfies the requirement, § 81-1 for an acute oral toxicity study in rats.

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MATERIALS:

1. Test compound: MTI (sample reference MTI BP1); Description: buff-colored solid; Lot. No.: Unspecified (CTL reference number was Y06823/001/001); Purity: 95.0% a.i. (As stated in a certificate of analysis in MRID 431387-23, for CTL Y06823/001/001).

2. Test animals: Species: rat, Strain: Wistar-derived, Alpk : APfSD; Age: Young adult; Weight (Day -1): 298-359 g (males) and 203-232 g (females); Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.; Acclimatization: a minimum of 6 days.

3. Animal husbandry: The rats were housed in suspended cages; a maximum of five rats in each cage, and the sexes were kept separately. The animals were allowed free access to food (Porton Combined Diet, Special Diet Services Ltd.) and given unlimited access to water via an automatic system. The rats were acclimatized a minimum of 6 days.

METHODS:

Rats were fasted for up to 24 hours before dosing. Test material in deionized water was administered orally (by gavage) to 5 rats/sex/dose at a rate of 10 ml/kg to give doses of 50, 100 or 500 mg MTI/kg b.w. Animals were observed for signs of toxicity within 2 hours after dosing and again between 4 and 7 hours after dosing and once daily (or twice daily if there were signs of toxicity) up to day 15. Rats were weighed on days -1, 1 (day of dosing), 3, 6, 8 and 15. Animals in extremis and animals surviving at the end of the study were necropsied and examined for macroscopic abnormalities.

The acute oral median lethal dose and the 95% confidence limits for the females were calculated from the mortality data by logistic regression using nominal dose values, confidence limits were calculated using a likehood ratio interval. Linear log-dose interpolation was used to estimate the acute oral median lethal dose for males.

RESULTS:

Doses and lethality are presented in Table 1.

Treatment-related clinical signs of toxicity included:

- Males: No signs were observed at 50 mg/kg. However, piloerection, reduced activity, upward curvature of the spine were observed at 100 mg/kg up to day 4. Reduced reflexes, prostration and death were observed at 500 mg/kg.

- Females: piloerection, upward curvature of the spine, staining (mouth or nose), and sides pinched in were observed at 50 and 100 mg/kg up to day 4. Prostration and death were observed at 500 mg/kg

Except for one female (No. 34, dosed at 100 mg/kg) that did not exceed its pre-dose weight at termination, all survivors had exceeded their pre-dose weights at termination.

Necropsy revealed blotchy livers and hemorrhagic stomach and intestines in all animals dosed with 500 mg/kg; these effects were not observed at lower doses.

Table 1. Mortality in Wistar-derived rats after a single oral dose of MTI[1].

Dose mg/kg	Males [deaths/dosed]	Females [deaths/dosed]
50	0/5	0/5
100	0/5	1/5[2]
500[3]	5/5	5/5
LD_{50}(CL)[4] =	224(100-500) mg/kg	168(92-306) mg/kg

[1] From p. 48 of the Study Report (MRID 431387-18).
[2] Found dead on day 2.
[3] One male was killed in extremis and the other animals were found dead within 3 hours of dosing.
[4] Approximate confidence limits for males; 95% confidence limits for females.

REGULATORY COMPLIANCE: Signed and dated quality assurance, GLP compliance and no confidentiality claim statements were present.

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011613

Reviewed by: Alberto Protzel, Ph.D. 5/22/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 5/23/95
Section III, Tox. Branch II(7509C)

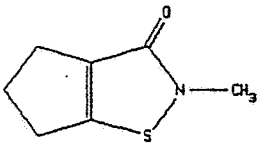
DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity in rats;
EPA Guideline 81-2

EPA IDENTIFICATION: EPA MRID No. 431387-15
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: PROMEXAL X50 (MTI formulation with 4.85% a.i.)

SYNONYMS/STRUCTURE: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)



STUDY NUMBER: Report No. CTL/P/3729; Study No. CR2980.

SPONSOR: ICI Americas Inc. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: PROMEXAL X50: Acute Dermal Toxicity to the Rat.

AUTHOR: P. Robinson

REPORT ISSUED: July 17, 1992 (Study Director signature dated July 6, 1990).

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID No. 431387-15), groups (SPF Wistar-derived, Alpk : APfSD) young albino rats (5/sex) were administered dermally PROMEXAL X50 (4.85% w/w MTI) at a dose of 2000 mg/kg b.w. (approximately 97 mg MTI/kg b.w.) to a shaved area of the dorsal trunk and covered with a 24 cm^2 patch for 24 hours. There were no signs of systemic toxicity. Slight to moderate erythema and edema were observed during the first 5-6 days of the observation period; slight desquamation was observed up to days 12-13 of the observation period. There were no male deaths and at most 1 female death.

LD_{50} : > 2000 mg/kg for both sexes.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity Category III and satisfies the requirement, § 81-2 for an acute dermal toxicity

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Category III and satisfies the requirement, § 81-2 for an acute dermal toxicity study in rats (for PROMEXAL X50).

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MATERIALS:

1. Test compound: PROMEXAL X50 (reference NBY 1919/24); Description: pale yellow liquid; Lot. No.: Unspecified (CTL reference number was Y06823/008); Purity: 4.85% w/w MTI [As stated in a certificate of analysis with reference No. NBY1457/48. The certificate of analysis was not included with the report]. The sample was used as supplied.

2. Test animals: Species: rat, Strain: Specific pathogen free (SPF) Wistar-derived albino, Alpk:APfSD; Age: Young adults at the beginning of the study; Weight (Day 1): 307-333 g (males) and 220-261 g (females); Source: Barriered Animal Breeding Unit. ICI Pharmaceuticals. Alderley Park, Macclesfield, Cheshire, UK.; Acclimatization: a minimum of 6 days.

METHODS:

Based on a preliminary range finding study, using several unspecified dose levels, a dose of 2000 mg of formulation/kg was selected for this study. Notice that at 4.85% w/w a.i. the applied dose in terms of active ingredient is 97 mg a.i./kg.

The hair was removed with clippers from an area (10 cm x 5 cm) on the dorso-lumbar region of 5 male and 5 female rats. The undiluted test sample was spread evenly using a 1 ml sterile disposable syringe. A volume of 2 ml/kg was applied. The test area was covered with a gauze patch (4 cm x 6 cm, 4-ply) and was kept under occlusive dressings for 24 hours.

Twenty four hours after treatment, the dressing was removed and the treated site was wiped with absorbent cotton soaked in clean warm water. The rats were observed for signs of systemic toxicity once between 1 and 4 hours after dosing and then once daily for systemic toxicity and skin irritation for up to 15 days. The animals were sacrificed and subjected to post mortem examination.

Signed and dated quality assurance and GLP compliance statements were present.

RESULTS:

No males died during the observation period. One female (No. 106) appears to have died on day 15 of the observation period: the body weight chart indicates that the animal was dead on the 15th day of observation, whereas, the pathology report indicates that the rat was killed at termination on day 15. No other female deaths were reported.

There were no signs of systemic toxicity. Slight to moderate erythema and edema were observed during the first 5-6 days of the observation period; slight desquamation was observed up to days 12-13 of the observation period.

All males exceeded their initial body weights. On female (No. 105) lost 1.6% of its initial body weight and another one (No. 104) retained its initial weight by day 15; there was no terminal body weight for female No. 106. At necropsy there were no abnormal findings, except for mottled lungs in one male (No. 5).

DISCUSSION:

A dose of 2000 mg of formulation/kg was selected for this study. At 4.85% w/w a.i. the applied dose in terms of active ingredient is 97 mg a.i./kg. There were no deaths among males and presumably no more than one death (on day 15) among females.

This material is classified in Toxicity Category III.

011613

Reviewed by: Alberto Protzel, Ph.D. 5/22/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 5/23/95
Section III, Tox. Branch II(7509C)

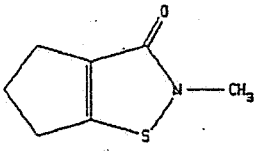
DATA EVALUATION REPORT

STUDY TYPE: Acute dermal toxicity in rats;
EPA Guideline 81-2

EPA IDENTIFICATION: EPA MRID No. 431387-19
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: Report No. CTL/P/2946.

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): Acute Dermal Toxicity to the Rat.

AUTHOR: J.C. McCall and A.M. Leah

REPORT ISSUED: May 16 1990 (Study Director signature dated May 3 1990).

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID No. 431387-19), groups (Wistar-derived, Alpk : APfSD) rats (7-9 weeks old, 2 males or 2 females/dose) were administered dermally MTI Technical as a paste at doses of 50, 200 or 400 mg/kg b.w. and as an aqueous formulation at doses of 200, 400 or 1000 mg/kg b.w. to a shaved area of the dorsal trunk and covered with a 24 cm^2 patch for 24 hours. With the paste at 200 mg/kg, both animals had large areas of skin necrosis and one rat was sacrificed on day 5 due to skin irritation; at 400 mg/kg both animals were sacrificed on day 3 due to skin irritation. With the aqueous formulation at 1000 mg/kg, both animals were sacrificed on day 5 due to skin irritation.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity

Category I and satisfies the requirement, § 81-2 for an acute dermal toxicity study in rats (for the Technical Material).

44/31

MATERIALS:

1. Test compound: MTI (sample reference MTI BP1); Description: buff-colored solid; Lot. No.: Unspecified (CTL reference number was Y06823/001/001); Purity: 95.0% (As stated in a certificate of analysis in MRID 431387-23, for CTL Y06823/001/001).

2. Test animals: Species: rat, Strain: Wistar-derived, Alpk : APfSD; Age: 7-9 weeks at the beginning of the study; Weight (Day -1): 252-329 g (males) and 177-203 g (females); Source: Barriered Animal Breeding Unit. ICI Pharmaceuticals. Alderley Park, Macclesfield, Cheshire, UK.; Acclimatization: a minimum of 6 days.

METHODS:

The study was conducted in two phases, the first phase tested the material as a paste. Because severe irritation was seen in the first phase at the higher doses, a second phase tested the material as a formulation in deionized water. The test groups are shown below in Table 1.

To apply the test material as a paste, the appropriate weight of test sample was moistened with 0.02-0.05 ml of deionized water and made into a paste. Groups of 2 male or 2 female rats (Table 1) had 50, 200 or 400 mg/kg of test material applied as a paste to a shaved area (50 cm^2) of the dorso-lumbar region. The test area was covered with a gauze patch (24 cm^2) and kept under occlusive dressings for 24 hours.

To apply the test material as a formulation, the test material was formulated in deionized water at an unspecified concentration. Groups of 2 male rats (Table 1) had 50, 400 or 1000 mg/kg of test material applied as a formulation using a 1 ml sterile syringe to a shaved area, as described above. As above, the test area was covered with a gauze patch (24 cm^2) and kept under occlusive dressings for 24 hours. Although the authors indicated that 2 ml/kg were dispensed no details were given to allow the reviewer to verify the accuracy of the dosing.

Twenty four hours after treatment, the dressing was removed and the treated site was wiped with absorbent cotton dipped in clean warm water. The rats were observed for signs of systemic toxicity once between 1 and 4 hours after dosing and then once daily for up to 9 days.

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Table 1. Test groups for acute dermal toxicity in rats.

	Number of Rats	
Dose level (mg/kg)	Males	Females
Applied as Paste[1]		
50	0	2
200	2	0
400	0	2
Applied as a Formulation[2]		
200	2	0
400	2	0
1000	2	0

[1] Test material was moistened with a small (0.020-0.050 ml) volume of deionized water. For example, for the 200 mg/kg level, rat #35 (329 g b.w.) received 65.8 mg of MTI moistened with 0.050 ml of deionized water.
[2] Test material was applied as a solution/suspension in deionized water. Enough volume of solution/suspension was added to achieve the required dose level. However, no details were given to verify the correctness of the dosing calculation.

Signed and dated quality assurance and GLP compliance statements were present.

RESULTS:

a. MTI applied as a paste:

50 mg/kg: there were slight to moderate signs of erythema and extreme signs of edema in one rat. Another rat showed slight erythema and moderate desquamation; this rat had urinary incontinence during days 1 and 2 of the study.

200 mg/kg: Both animals had large areas of skin necrosis. One rat was sacrificed on day 5 due to skin irritation.

400 mg/kg: Both animals had edema (rated extreme) and large areas of skin necrosis (rated equivocal). One rat had urinary incontinence during days 1-2 of the study; the other rat showed slight curvature of the spine on days 2 and 3. Both animals were sacrificed on day 3 due to skin irritation.

b. MTI applied as a formulation in deionized water:

200 mg/kg: Both rats showed moderate to slight erythema, in one case

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lasting up to day 9 in the other case up to day 6. One rat had large areas of necrosis (probably blanching). Both rats had moderate to extreme edema up to day 5.

400 mg/kg: Both rats showed moderate to extreme erythema and extreme edema. Both rats showed moderate curvature of the spine on days 2 and 3. Both animals were sacrificed at termination on day 9.

1000 mg/kg: Extreme edema observed in both animals. White and black necrosis observed in both rats. Both rats had upward curvature of the spine and were sacrificed on day 5 due to skin irritation.

DISCUSSION:

At 200 mg/kg (material applied as a paste), both rats had extensive areas of necrosis and one rat had to be sacrificed on day 5 due to skin irritation. At 400 mg/kg (material applied as a paste) both rats had to be sacrificed on day 3, prior to termination of the study due to skin irritation. Likewise, both rats had to be sacrificed when the material was formulated in water and given at 1000 mg/kg.

The test material is classified in Toxicity Category I due to the severity of the skin effects.

011613

Reviewed by: Alberto Protzel, Ph.D.
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D.
Section III, Tox. Branch II(7509C)

Alberto Protzel 5/30/95
James N. Rowe 5/30/95

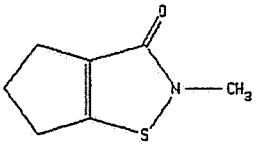
DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation in Rabbits;
EPA Guideline 81-4

EPA IDENTIFICATION: EPA MRID No. 431387-16
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: PROMEXAL W50

SYNONYMS/STRUCTURE: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)



STUDY NUMBER: Report No. CTL/L/5062.

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: PROMEXAL W50: In Vitro Ocular Irritation Assessment.

AUTHOR: P. Robinson

REPORT ISSUED: December 23,1992 (Study Director signature dated December 21, 1992)

EXECUTIVE SUMMARY: In an in vitro primary eye irritation study (MRID 431387-16), two isolated eyes from New Zealand White rabbits (source unspecified) were exposed for 10 seconds to 0.1 ml undiluted PROMEXAL W50. Following rinsing of the eyes with saline, corneal swelling was determined at 0.5, 1, 2, 3, 4, and 5 hours after dosing. Swelling increased with time, from 10.5% at 0.5 hours to 86.8% at 5 hours. Based on the large corneal swelling (> 15%), it was concluded that PROMEXAL W50 is likely to be a severe ocular irritant in vivo.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity Category I and satisfies the requirement, § 81-4 for a primary eye irritation study.

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MATERIALS:

1. Test compound: PROMEXAL X50 (sample reference NBY 1919/24); Description: pale yellow liquid (based on the description given in MRID No. 431387-14 for a PROMEXAL W50 sample with sample reference NBY 1919/24); Lot. No.: Unspecified (CTL reference number was Y06823/008); Purity: Unspecified. [However, a purity of 4.85% w/w MTI was given in MRID No. 431387-14 for a PROMEXAL W50 sample with sample reference NBY 1919/24]. The sample was tested as supplied.

2. Test animals: This test was done in vitro using the isolated eyes of New Zealand White albino rabbits (Age and sex unspecified).

METHODS:

1. Introduction: The test method was based on a modified form of the in vitro isolated eye test [Burton et al. The in vitro assessment of severe eye irritants. Fd. Cosmet. Toxicol. 19: 471-480 (1981)].

The present test is part of a two-tiered battery of in vitro tests. The test battery comprises first a cytotoxicity test, the K562 cell assay. Materials that reduce cell viability to 85% or less in the cytotoxicity test proceed to tier 2 of the battery, the isolated rabbit eye test (IET). The IET is done to evaluate the potential of the test material to cause severe eye effects in vivo. A threshold value has been defined (15% or greater corneal swelling in the IET up to 5 hours) as indicative of a potential of the test material to cause severe effects in vivo.

2. Test method (IET): Details of the method appear in attachment 1. In brief, New Zealand White albino rabbits were used for this assay. Each eye was carefully removed from the eye socket and then mounted in a vertical position in a perspex clamp. A total of two eyes was used for this experiment.

Saline was dripped onto the upper margin of the cornea in order to irrigate the whole surface of the cornea. The percent of corneal swelling after contact with saline was measured prior to treatment with test material. Eyes that swelled more than 4% were rejected. Using a 1 ml syringe, 0.1 ml of the test material was applied to the corneal surface and left in contact for 10 seconds. The test material was removed by rinsing with at least 20 ml of warm saline. Measurements of corneal thickness were taken at 0.5, 1, 2, 3, 4, and 5 hours after dosing. Corneal swelling was then determined for both eyes and the standard deviation was calculated. A chemical causing the eyes to swell more than 15% in vitro is considered to have a potential to cause severe ocular irritation in vivo.

RESULTS:

As summarized below in Table 1, the test chemical produced a mean percent swelling of 86.8% over the 5 hour observation period. The authors concluded, that on the basis of the large corneal swelling observed in the isolated eye

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test, PROMEXAL W50 is likely to be a severe ocular irritant in vivo.

On the basis of the potential for severe ocular irritation in vivo, as evaluated in vitro with IET, and in the absence of a standard in vivo Guideline 81-4 study to show otherwise, PROMEXAL X50 is placed in toxicity category I for eye irritation.

~~50~~ 37

Table 1. Mean percentage corneal swelling (From p. 10 of the Study Report, MRID 431387-16)

	Time (hours)					
	0.5	1.0	2.0	3.0	4.0	5.0
Mean % corneal swelling	10.5	13.2	18.4	42.1	68.4	86.8
± S. deviation	0	0	0	0	0	3.7

Attachment 1
Test Method (from pp. 6-7 of the Study Report. MRID 431387-16)

EPA Reg. NO. 10182-385

Page_____ is not included in this copy.

Pages 40 through 41 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

011613

Reviewed by: Alberto Protzel, Ph.D. 5/23/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 5/23/95
Section III, Tox. Branch II(7509C)

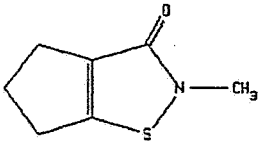
DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation in Rabbits;
EPA Guideline 81-4

EPA IDENTIFICATION: EPA MRID No. 431387-20
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: Report No. CTL/P/2811.

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): Eye Irritation to the Rabbit.

AUTHOR: A. Barlow and A.M. Leah

REPORT ISSUED: March 13 1990 (Study Director signature dated February 22, 1990).

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 431387-20), one male New Zealand White rabbit from Mellor Rabbits, Chadderton Heights, Chadderton, Nr Oldham, Greater Manchester, UK. was treated in the conjunctival sac with 50 mg of MTI Technical. Approximately one hour after application, the rabbit was seen to be in distress. Signs of ocular irritation included severe redness and chemosis of the conjunctiva, a slight discharge and moderate erythema of the eyelids. The animal was sacrificed 1.5 hours after application.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity Category I and satisfies the requirement, § 81-5 for a primary eye irritation study in rabbits (for the Technical Material).

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MATERIALS:

1. Test compound: MTI (sample reference MTI BP1); Description: buff-colored solid; Lot. No.: Unspecified (CTL reference number was Y06823/001/001) Purity: 95.0% (As stated in a certificate of analysis in MRID 431387-23, for CTL Y06823/001/001). The test material was used as supplied.

2. Test animals: Species: Rabbit; Strain: New Zealand White; Age: Young adult; Weight (At the beginning of the study): 3672 g (only 1 male was used); no females were used in this study. Source: Mellor Rabbits, Chadderton Heights, Chadderton, Nr Oldham, Greater Manchester, UK. Acclimatization: a minimum of 6 days.

METHODS:

One male rabbit (without apparent eye effects or irritation) received a single dose of approximately 50 mg (of an intended 100 mg) of the test material in the conjunctival sac of the left eye. The eyelids of the treated eye were gently held together for 1-2 seconds; the other eye was left untreated and served as a control. Immediately after application of the test sample, an assessment of the initial pain reaction was made using a six-point scale. The eyes were examined for irritation using a Draize scale, approximately one-hour after application.

RESULTS:

Application of MTI (50 mg, one half of the intended 100 mg) into the eye produced a moderate initial pain reaction (Grade 3 on a scale of 0-5) and immediate closure of the eye. The remaining 50 mg were not applied.

Approximately one hour after application, the rabbit was seen to be in distress. Signs of ocular irritation included severe redness (Grade 3, severe) and chemosis (Grade 4, severe) of the conjunctiva, a slight discharge (Grade 1, slight) and moderate erythema of the eyelids. The animal was sacrificed 1.5 hours after application.

As severe effects were seen no further animals were dosed. The test material was considered by the authors to be at least an extremely severe irritant of the rabbit eye.

This chemical is classified in Category I for eye irritation.

011613

Reviewed by: Alberto Protzel, Ph.D. Alberto Protzel 5/22/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. James N. Rowe 5/23/95
Section III, Tox. Branch II(7509C)

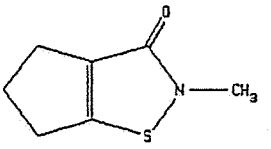
DATA EVALUATION REPORT

STUDY TYPE: Primary Skin Irritation in Rabbits;
EPA Guideline 81-5

EPA IDENTIFICATION: EPA MRID No. 431387-21
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: Report No. CTL/P/2810.

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): Skin Irritation to the Rabbit.

AUTHOR: A. Barlow and A.M. Leah

REPORT ISSUED: March 13 1990 (Study Director signature dated February 22, 1990).

EXECUTIVE SUMMARY: In a primary skin irritation study (MRID 431387-21), 1 in^2 areas of intact skin of New Zealand White rabbits (3 males) from Mellor Rabbits, Chaddertton Heights, Greater Manchester, UK were exposed to 500 mg of MTI Technical moistened with 0.1 ml deionized water for 4 hours. Very slight to well defined erythema (Grades 1-2) and well defined edema (Grade 2) were seen in all animals at 30-60 minutes after patch removal. No erythema or edema (Grades = 0) were seen at 72 hours after dosing.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity Category IV and satisfies the requirement, § 81-5 for a primary skin irritation study in rabbits (for the Technical Material).

MATERIALS:

1. Test compound: MTI (sample reference MTI BP1); Description: buff-colored solid; Lot. No.: Unspecified (CTL reference number was Y06823/001/001); Purity: 95.0% (As stated in a certificate of analysis in MRID 431387-23, for CTL Y06823/001/001).

2. Test animals: Species: Rabbit; Strain: New Zealand White; Age: Young adult; Weight (At the beginning of the study): 3770-3954 g (males), no females were used in this study. Source: Mellor Rabbits, Chaddertton Heights, Chadderton, Nr Oldham, Greater Manchester, UK. Acclimatization: a minimum of 6 days.

METHODS:

The hair on the left flank of 3 male rabbits was removed using veterinary clippers from an area approximately 7 cm x 13 cm. The test sample (approximately 500 mg) was moistened with 0.1 ml of deionized water and applied to the test site (approximately 2.5 cm x 2.5 cm) using a metal spatula. The area was covered with surgical gauze (2.5 cm x 2.5 cm, 8 ply) and secured with surgical tape. The area was then covered with impermeable rubber sheeting and secured with surgical tape. Four hours after treatment, dressings were removed and test areas were wiped with absorbent cotton dipped in clean warm water and gently dried with clean tissue paper. Treated sites were scored for erythema and edema at 4.5, 24, 48, and 72 hours after removal of the patch.

Signed and dated quality assurance and GLP compliance statements were present.

RESULTS:

Very slight to well defined erythema (Grades 1-2) were seen in all animals at 30-60 minutes after patch removal. Very slight (Grade 1) erythema was seen in all animals at days 1 and 2 after patch removal. No erythema was seen on day 3 after patch removal.

Well defined edema (Grade 2) was seen in all animals at 30-60 minutes after patch removal. Very slight (Grade 1) edema was seen in all animals at days 1 and 2 after patch removal. No edema was seen on day 3 after patch removal.

A small scab was seen on the edge of the application site in one animal (animal 13) on days 3 and 4 after patch removal.

DISCUSSION:

No erythema or edema (Grades = 0) were seen at 72 hours after dosing. It is noted only three rabbits were used for this even though Guideline 81-5 requires at least 6 animals. Because the effects were nearly identical in all three rabbits tested, it appears that the use of three more rabbits would not add significantly to the observed results.

This study is placed in Category IV for primary skin irritation.

58 45

011613

Reviewed by: Alberto Protzel, Ph.D. 5/22/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 5/23/95
Section III, Tox. Branch II(7509C)

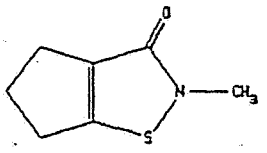
DATA EVALUATION REPORT

STUDY TYPE: Primary Skin Irritation in Rabbits;
EPA Guideline 81-5

EPA IDENTIFICATION: EPA MRID No. 432250-02
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: PROMEXAL W50 (4.85% w/w formulation of MTI)

SYNONYMS/STRUCTURE: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)



STUDY NUMBER: Report No. CTL/P/3773.

SPONSOR: ICI Americas, Inc. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: PROMEXAL W50: Skin Irritation to the Rabbit.

AUTHOR: P. Robinson

REPORT ISSUED: August 21 1992 (Study Director signature dated July 28, 1992).

EXECUTIVE SUMMARY: In a primary skin irritation study (MRID 432250-02), 1 in^2 areas of intact skin of New Zealand White rabbits (6 males) from the Conventional Animal Breeding Unit. Alderley Park, Macclesfield, Cheshire, (UK) were exposed to 0.5 ml of undiluted PROMEXAL W50 (4.85% w/w MTI) for 4 hours. Severe erythema was observed in 5/6 rabbits, together with severe edema in all rabbits at 30-60 minutes after dosing. On day 3, 1/6 rabbits had severe erythema and 4/6 rabbits had moderate to severe erythema. On day 3, 3/6 rabbits had severe edema and 2/6 had moderate to severe edema. One rabbit still had severe erythema and another one still had severe edema on day 7. Erythema persisted in two rabbits up to day 21 after treatment.

The study is classified as Core Minimum Data (Acceptable) with a Toxicity Category II and satisfies the requirement, § 81-5 for a primary skin irritation

Category II and satisfies the requirement, § 81-5 for a primary skin irritation study in rabbits (for PROMEXAL X50).

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MATERIALS:

1. Test compound: PROMEXAL X50 (reference NBY 1919/24); Description: pale yellow liquid; Lot. No.: Unspecified (CTL reference number was Y06823/008); Purity: 4.85% w/w MTI [As stated in a certificate of analysis with reference No. NBY1457/48. The certificate of analysis was not included with the report]. The sample was used as supplied.

2. Test animals: Species: Rabbit; Strain: New Zealand White; Age: Young adult; Weight (At the beginning of the study): 3258-4343 g (males), no females were used in this study. Source: Conventional Animal Breeding Unit. Alderley Park, Macclesfield, Cheshire, UK.; Acclimatization: a minimum of 6 days.

METHODS:

The hair on the left flank of 6 male rabbits was removed using veterinary clippers from an area approximately 7 cm x 13 cm. The undiluted test sample (0.5 ml) was applied to the test site (approximately 2.5 cm x 2.5 cm) using a polypropylene syringe. The treated area was covered with surgical gauze (2.5 cm x 2.5 cm, 8 ply) and secured with surgical tape. The area was then covered with impermeable rubber sheeting and secured with impermeable polyethylene tape. Four hours after treatment, the dressings were removed and the test areas were wiped with absorbent cotton dipped in clean warm water and gently dried with clean tissue paper. Treated sites were scored for erythema and edema at 30-60 minutes and at 1, 2, and 3 days after removal of the patch using a Draize scale). Skin samples were obtained on day 21 from the test site plus controls from the opposite flank from two animals (Nos. 9 and 10) for histological examination.

Signed and dated quality assurance and GLP compliance statements were present.

RESULTS:

Severe erythema (Grade 4) was seen in most rabbits (5/6) at 30-60 minutes and on day 1. On day 3, 1/6 rabbits had severe erythema and 4/6 rabbits had moderate to severe erythema. On day 7, one of six rabbits (animal 18) still had severe (Grade 4) erythema, scores for the other rabbits ranged from slight (Grade 1) to moderate-to-severe (Grade 3). On Day 21 one rabbit (No. 9) had well defined erythema (Grade 2) and another rabbit (No. 10) had slight erythema (Grade 1).

Severe edema (Grade 4) was seen in all rabbits at 30-60 minutes and on days 1 and 2 after dosing. On day 3, 3/6 rabbits had severe edema and 2/6 had moderate to severe edema. On day 7, one of 6 rabbits (animal 18) still had severe edema (Grade 4), scores for the other rabbits were either 0 or 1. No edema was seen by day 12.

Histological examination of the test areas of animals Nos. 9 and 10 revealed minimal diffuse hyperkeratosis and acanthosis, minimal multifocal inflammatory cell infiltration, and minimal to slight subepithelial fibrosis. Effects were limited to the epidermis and the dermal/epidermal junction; deeper dermal structures were not affected.

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DISCUSSION

On day 3, 1/6 rabbits had severe erythema and 4/6 rabbits had moderate to severe erythema. On day 3, 3/6 rabbits had severe edema and 2/6 had moderate to severe edema. One rabbit still had severe erythema and another one still had severe edema on day 7. Erythema persisted in two rabbits up to day 21 after treatment.

The test material is placed in Toxicity Category II for primary skin irritation.

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011613

Reviewed by: Alberto Protzel, Ph.D. 6/12/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 7/10/95
Section III, Tox. Branch II(7509C)

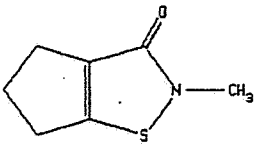
DATA EVALUATION REPORT

STUDY TYPE: Skin sensitization in Guinea Pigs;
EPA Guideline 81-6

EPA IDENTIFICATION: EPA MRID No. 431387-22
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: Report No. CTL/P/2960.

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): Skin Sensitization to the Guinea Pig.

AUTHOR: D. Lees

REPORT ISSUED: August 6, 1990 (Study Director signature dated July 20, 1990).

EXECUTIVE SUMMARY: In a dermal sensitization study (a Buehler test, MRID No. 431387-22), 20 albino Porcellus Dunkin Hartley female guinea pigs from Harlan Porcellus, Firgrove Farm, Heathfield, Sussex, UK. were induced dermally with a 3% (w/v) preparation of the test material in deionized water. Induction was done in a total of 3 six-hour exposures (the interval between exposures was 7 days)to the test material. Two weeks after the final induction, the test animals were challenged dermally with 1% (w/v) and 0.3% (w/v) preparations of the test material in deionized water. Challenge with the 1% (w/v) preparation produced a net percentage response of 70%, which falls in the strong sensitizer category. Challenge with the 0.3% (w/v) preparation produced a net percentage response of 90%, which falls in the extreme sensitizer category. Technical MTI was found to be an extreme sensitizer under the conditions of this test.

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The study is classified as Minimum and satisfies the requirement, § 81-6 for a dermal sensitization study in Guinea pigs.

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MATERIALS:

1.(a) Test compound: MTI (sample reference MTI BPI); Description: buff-colored solid; Lot. No.: Unspecified (CTL reference number was Y06823/001); Purity: 95.0% (As stated in a certificate of analysis in MRID 431387-23, for CTL Y06823/001/001).

(b) Positive Control: Formaldehyde. A 40% w/v aqueous solution was used. The sample was given the CTL reference No. Y00446/002/004 and was used as a 10% and a 30% (w/v) solution in deionized water. Sensitizing capacity of the formaldehyde solution are assessed periodically; the most recent positive controls were used in this study.

(c) Vehicles: Deionized water.

2. Test animals: Species: Guinea Pig (females); Strain: Albino Porcellus Dunkin Hartley Strain; Age: Unspecified; Weight (Main study, at the beginning): 447-620 g (females), no males were used in this study; Weight (Positive controls; at the beginning): 366-453 g (females); Source: Harlan Porcellus, Firgrove Farm, Heathfield, Sussex, UK. Acclimation: a minimum of 6 days.

METHODS:

Sensitization studies were conducted using Buehler's Method.

1. Irritation Screening Studies:

Pre-induction and pre-challenge irritation screening studies were performed to determine suitable concentrations of the test material for use in the induction and challenge phases of the main study, respectively.

On the basis of the preliminary screening study, a 3% (w/v) concentration of the test material was selected for induction in the main study. For the pre-challenge screen, 1% (w/v) and 0.3% (w/v) preparations of the test material were applied to each of two female guinea pigs. There was only mild and transient irritation with the 1% solution. Thus, 1% (w/v) and 0.3% (w/v) preparations of the test material were used in the challenge phase of the main study.

2. Induction of Sensitization:

The dosing groups used in the main study are shown in Table 1. An area approximately 5 cm x 5 cm on the scapular region of each animal was clipped free of hair prior to treatment. Test Material was administered as 0.4 ml of a 3% (w/v) preparation in deionized water. The 3% preparation of the test material or the Vehicle (deionized water) was applied to a lint pad (approx. 2 cm x 2 cm) which was covered with a patch of adhesive tape, and held in place by adhesive elastic bandage secured by a piece of PVC tape. The patches were removed after a 6-hour exposure. The induction procedure was repeated at the same site, during the following 2 weeks, for a total of 3 six-hour exposures (the interval between exposures was 7 days). Due to the severity of the irritation response, the concentration of test material applied at the third induction was lowered to 1%

65 52

(w/v). Positive control was administered as a 30% and 10% (w/v) dilution of 40% (w/v) aqueous solution of formaldehyde as described above.

Table 1. Study groups for dermal sensitization studies with MTI Technical.

Group No.	Applied Material	Number of Animals
1.	Uninduced controls for test material[a]	10
2.	Test Material	20
3.	Uninduced controls for positive control[b]	10
4.	Positive control[c] (Formaldehyde solution))	20

[a] Noninduced; treated with test material during challenge phase only.
[b] Noninduced; treated with positive control [40%, w/v, formaldehyde solution in deionized water applied as 30% and 10% (w/v) solutions] during the challenge phase only.
[c] The capacity of the guinea pig strain to respond to a known sensitizing agent is assessed periodically at the testing facility, using formaldehyde. The positive control data included with this study are the most recent positive control data available at the testing facility.

3. Challenge Phase:

The Guinea pigs were challenged two weeks after the final induction. An area of approximately 15 cm x 5 cm on one or both flanks of each animal was clipped free of hair. Test animals and uninduced controls (Groups 1 and 2, respectively, in Table 1) were challenged with a 1% (w/v) preparation (≈ 0.1-0.2 ml) and a 0.3% preparation (≈ 0.1-0.2 ml) of the test material in deionized water. The application pad was held in place by adhesive impermeable polyethylene tape. The dressings were removed after 6 hours.

Erythematous reactions were quantified at 24 and 48 hours after removal of the dressings using the following 4-point scale:

0 - no reaction
1 - scattered mild redness
2 - moderate diffuse redness
3 - intense redness and swelling

The evaluate the sensitization response, the percent of the control animals that responded was subtracted from the percentage of test animals that responded and the percent net response was rated according to the following scale:

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Net Score	Rating
0	Not a sensitizer
1-8	Weak sensitizer
9-28	Mild sensitizer
29-64	Moderate sensitizer
65-80	Strong sensitizer
81-100	Extreme sensitizer

Positive controls and their uninduced controls (Groups 3 and 4 in Table 1) were challenged with a 30% (w/v) solution of a formaldehyde solution (40% w/v in deionized water). No additional data on sample application for positive controls was available to the reviewer because the page containing the information was missing in the report.

Signed and dated quality assurance, no data confidentiality and GLP compliance statements were present.

RESULTS:

1. Irritation screening:

Signs of severe irritation were seen in the test animals during the induction phase. No signs of irritation were seen in the uninduced controls. Signs of slight to moderate irritation were seen in the positive controls. No signs of irritation were seen in the respective control animals.

2. Challenge phase:

Results from the challenge with test material 1% (w/v) and 0.3% (w/v) appear in Attachment 1 (for test animals) and Attachment 2 (for uninduced controls). For the 1% solution at 24 and 48 hours, the severity was clearly higher in the test animals (scores of 1-3) than in the uninduced controls (scores of 0-1). The net percentage response was 70%, which falls in the strong sensitizer category. For the 0.3% solution at 24 and 48 hours, the severity was still higher in the test animals (scores of 0-2) than in the uninduced controls (scores of 0). The net percentage response was 90%, which falls in the extreme sensitizer category.

The positive control, a formaldehyde solution, gave a net percent response of 68%, which places it in the category of strong sensitizer.

DISCUSSION:

The test material as a 0.3% (w/v) solution in deionized water elicited a extreme skin sensitization response in previously induced guinea pigs. In a positive control study, challenge with a 30% (w/v) solution of a formaldehyde solution [40% in water] elicited a strong sensitization response in previously-induced guinea pigs. Missing details on the application of the formaldehyde solution used as a positive control do not invalidate this study, since the test compound appears to be clearly positive in this test and an adequately positive response was observed in the positive controls.

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Attachment 1
MTI Technical. Challenge Results in Test Animals.
From pp. 22 of the Study Report (MRID 431387-22)

EPA Reg. No. 10182-385

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011613

Reviewed by: Alberto Protzel, Ph.D. 5/30/95
Section III, Tox. Branch II(7509C)
Secondary reviewer: James N. Rowe, Ph.D. 5/30/95
Section III, Tox. Branch II(7509C)

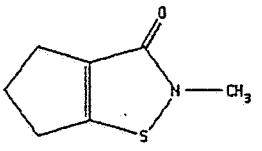
DATA EVALUATION REPORT

STUDY TYPE: Skin sensitization in Guinea Pigs;
EPA Guideline 81-6

EPA IDENTIFICATION: EPA MRID No. 431387-17
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: PROMEXAL X50 (4.85% w/w formulation of MTI)

SYNONYMS/STRUCTURE: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)



STUDY NUMBER: Report No. CTL/P/3844

SPONSOR: ICI Americas Inc. ICI Specialties. Wilmington, DE 19897.

TESTING FACILITY: ICI Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: PROMEXAL X50: Skin Sensitization to the Guinea Pig.

AUTHOR: D. Lees, A.M. Leah

REPORT ISSUED: December 9, 1992 (Study Director signature dated November 24, 1992).

EXECUTIVE SUMMARY: In a dermal sensitization study (a Buehler test, MRID No. 431387-17), 20 albino Alpk:Dunkin Hartley female guinea pigs from the Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK, were induced dermally with undiluted PROMEXAL X50. Induction was done in a total of 3 six-hour exposures (the interval between exposures was 7 days) to the test material. Two weeks after the final induction, the test animals were challenged dermally with 30%, 10%, 3% and 1% w/v dilutions of the test material in deionized water. Challenge with the 30% and 10% (w/v) dilutions produced net percentage responses of 63 and 44%, respectively, which fall in the moderate sensitizer category. Challenge with the 3% (w/v) dilution produced a net percentage response of 5%, which falls in the weak sensitizer category; the 1% solution did not elicit any sensitization response. PROMEXAL X50 was found to

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be a moderate sensitizer under the conditions of this test.

The study is classified as Minimum and satisfies the requirement, § 81-6 for a dermal sensitization study in Guinea pigs.

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MATERIALS:

1. (a) Test compound: PROMEXAL W50 (sample reference NBY 1919/43); Description: light brown/orange liquid; Lot. No.: Unspecified (CTL reference number was Y06823/009); Purity: 4.85% w/w MTI [As stated in a certificate of analysis with reference No. NBY 1919/5. The certificate of analysis was not included with the report]. The sample was used as preparations in deionized water.

(b) Positive Control: Formaldehyde. A 40% w/v aqueous solution was used. The sample was given the CTL reference No. Y00446/002 and was used as a 30% (w/v) solution in deionized water. Sensitizing capacity of the formaldehyde solutions are assessed periodically; the most recent positive controls were used in this study.

(c) Vehicle: Deionized water.

2. Test animals: Species: Guinea Pig (females); Strain: Albino Alpk:Dunkin Hartley; Age: young adult; Weight (Main study, at the beginning): 465-733 g (females), no males were used in this study; Weight (Positive controls; at the beginning): 491-650 g (females); Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK. Acclimation: a minimum of 6 days.

METHODS:

Sensitization studies were conducted using Buehler's Method.

1. Irritation Screening Studies:

Dose levels for this study were selected on the basis of data from previous, unspecified, studies on the test sample carried on at the test laboratory. The undiluted test sample was selected for the induction phase and a 30% w/v preparation of the test sample in deionized water (highest non-irritant concentration), together with a range of lower concentrations was selected for the challenge phase.

2. Induction of Sensitization:

The dosing groups used in the main study are shown in Table 1. An area approximately 5 cm x 5 cm on the scapular region of each animal was clipped free of hair prior to treatment. Test Material was administered as 0.4 ml of the undiluted test material that was applied to a lint pad (approx. 2 cm x 2 cm) which was covered with a patch of adhesive tape, and held in place by adhesive elastic bandage secured by a piece of PVC tape. Uninduced controls were treated with the bandage alone. The patches were removed after a 6-hour exposure. The induction procedure was repeated at the same site, one week later and again a further weeks, for a total of 3 six-hour exposures. Due to the severity of the irritation response, the third application used a new site for some animals. Positive control was administered as a 30% (w/v) dilution of 40% (w/v) aqueous solution of formaldehyde as described above.

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Table 1. Study groups for dermal sensitization studies with PROMEXAL W50.

Group No.	Applied Material	Number of Animals
1.	Uninduced controls for test material[a]	10
2.	Test Material	20
3.	Uninduced controls for positive control[b]	10
4.	Positive control[c] (Formaldehyde solution))	20

[a] Noninduced; treated with test material (30%, 10%, 3% or 1% w/v dilutions in deionized water) during challenge phase only.
[b] Noninduced; treated with positive control [40%, w/v, formaldehyde solution in deionized water applied as 30% (w/v) dilutions] during the challenge phase only.
[c] The capacity of the guinea pig strain to respond to a known sensitizing agent is assessed periodically at the testing facility, using formaldehyde. The positive control data included with this study are the most recent positive control data available at the testing facility.

3. Challenge Phase:

The Guinea pigs were challenged two weeks after the final induction. An area of approximately 15 cm x 5 cm on both flanks of each animal was clipped free of hair. Each animal from the test and uninduced control groups (Groups 1 and 2, respectively, in Table 1) was challenged with 30%, 10%, 3% and 1% w/v dilutions of the test material in deionized water. The application pad was held in place by adhesive impermeable polyethylene tape. The dressings were removed after 6 hours.

Erythematous reactions were quantified at 24 and 48 hours after removal of the dressings using the following 4-point scale:

0 - no reaction
1 - scattered mild redness
2 - moderate diffuse redness
3 - intense redness and swelling

The evaluate the sensitization response, the percent of the control animals that responded was subtracted from the percentage of test animals that responded and the net response was rated according to the following scale:

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Net Score	Rating
0	Not a sensitizer
1-8	Weak sensitizer
9-28	Mild sensitizer
29-64	Moderate sensitizer
65-80	Strong sensitizer
81-100	Extreme sensitizer

Positive controls and their uninduced controls (Groups 3 and 4 in Table 1) were challenged with a 30% (w/v) dilution of a formaldehyde solution (40% w/v in deionized water). No additional data on sample application for positive controls was available to the reviewer.

Signed and dated quality assurance, no data confidentiality and GLP compliance statements were present.

RESULTS:

1. Induction phase:

Signs of moderate to severe irritation were seen in the test animals during the induction phase. One animal (No. 75) was killed before challenge due to the severity of the response. Another animal (No. 62) was found dead; its death is not considered related to treatment, since there were no prior clinical signs of toxicity. Signs of moderate irritation were seen in the positive controls. No signs of irritation were seen in the respective control animals.

2. Challenge phase:

Results from the challenge with PROMEXAL W50 at dilutions of 30%, 10%, 3% and 1% w/v in deionized water appear in Attachment 1 (for test animals) and Attachment 2 (for uninduced controls).

For the 30% (w/v) dilution at 24 and 48 hours, the severity was clearly higher in the test animals (scores of 0-3) than in the uninduced controls (scores of 0-1, all 0 at 48 hours). The net percentage response was 63%, which falls in the moderate sensitizer category.

For the 10% dilution at 24 and 48 hours, the severity was still higher in the test animals (scores of 0-2) than in the uninduced controls (scores of 0). The net percentage response was 44%, which falls in the moderate sensitizer category.

For the 3% dilution, all induced animals scored 0 at 48 hours and only one had any erythema at 24 hours (score of 1). The net percentage response was 5%, which falls in the weak sensitizer category. There was no erythematous response in any of the animals challenged with the 1% (w/v) dilution.

The positive control, a formaldehyde solution, gave a net percent response of 55%, which places it in the category of moderate sensitizer.

DISCUSSION:

PROMEXAL W50 as 30 or 10% (w/v) dilutions in deionized water elicited a moderate skin sensitization response in previously induced guinea pigs. Additionally, PROMEXAL W50 as a 3% (w/v) dilutions in deionized water elicited a weak skin sensitization response in previously induced guinea pigs; there was no response when the challenge was conducted with a 1% (w/v) dilution of PROMEXAL W50. In a positive control study, challenge with a 30% (w/v) solution of a formaldehyde solution [40% in water] elicited a moderate sensitization response in previously-induced guinea pigs.

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Attachment 1
PROMEXAL W50. Challenge Results in Test Animals.
From pp. 22 and 23 of the Study Report (MRID 431387-17)

EPA Reg. NO 10182-385

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____ Description of quality control procedures.

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____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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Attachment 2
PROMEXAL W50. Challenge Results in Control Animals.
From pp. 24 and 25 of the Study Report (MRID 431387-17)

EPA Reg. No. 10182-385

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011613

Reviewed by: Alberto Protzel, Ph.D.
Review Section III, Toxicology Branch II(7509C) 2/13/95
Secondary Review by: James N. Rowe, Ph.D.
Review Section III, Toxicology Branch II(7509C) 2/14/95

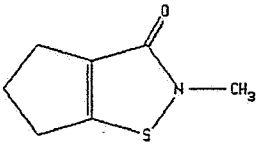
DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral
Species: Rat
EPA Guideline 82-1

EPA IDENTIFICATION: EPA MRID No. 431387-24
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: PR0903 (Report No. CTL/P/3858).

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: Zeneca Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): 90 Day Feeding Study in Rats.

AUTHOR: N. J. Rattray

REPORT ISSUED: August 27 1993 (Study Director signature dated July 23 1993).

EXECUTIVE SUMMARY: In a 13-week subchronic feeding study MTI was administered to Alpk:APfSD (Wistar derived) rats of both sexes for a period of 90 days in the diet at dose levels of 0, 50, 250, or 1000 ppm, corresponding to mean MTI intake of 0, 4.1, 20.7 or 83 mg/kg/day (in males) and 0, 4.6, 23.2, or 93.0 mg/kg/day (in females). Statistically significantly decreased body weights were observed at 1000 ppm in males throughout the treatment period (e.g. to 84.8% of controls at 14 weeks). Mean body weight gains at 250 and 1000 ppm in males were reduced to 95.2 and 79.3% of controls for weeks 1-14. Mean food efficiency values for high dose males were statistically significantly decreased for weeks 1-13 (to 89.7% of controls). Statistically significantly decreased body weights were observed at 250 and 1000 ppm in females throughout the treatment period (e.g. to 94.5 and 87.8% of controls at 14 weeks for 250 and 1000 ppm, respectively).

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Likewise, mean body weight gains decreased in dose-related fashion to 89.1% and 68.7% of controls at 250 and 1000 ppm, respectively, at 1-5 weeks and to 92.6% and 76.7% of controls, at 250 and 1000 ppm, respectively, for weeks 1-14. Mean food efficiency values for weeks 1-4 decreased with increasing dose, reaching statistical significance at 250 and 1000 ppm, at 91.3 and 76.5% of controls, respectively. There were no apparent toxicologically significant findings in clinical chemistry, hematology, or macroscopic and microscopic pathology. The LEL of 250 ppm (♂: 20.7 mg/kg/day; ♀: 23.2 mg/kg/day) is based on dose-related and statistically significant, decreases in food efficiency for females at 250 and 1000 ppm for weeks 1-4), coupled to dose-related decreases in body weight gain at 250 and 1000 ppm for weeks 1-4). The NOEL is 50 ppm (♂: 4.1 mg/kg/day; ♀: 4.6 mg/kg/day).

This study is classified as Core Minimum. This study satisfies the requirement, § 82-1 for a subchronic oral toxicity in rodents.

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- Decreases in body weight gain and food efficiency:
  In males, there were decreases in food efficiency (down to 88.1% and 31.3% of controls at 400 and 1000/1250 ppm, respectively) during the first week and decreases in bodyweight gain during the first four days at 400 ppm and throughout the experiment at 1000/1250 ppm (e.g. down to 70% of controls on day 29 at 1000/1250 ppm). In the male recovery group (1000/1250 ppm), although body weight gains were 65.3% of controls on day 29, they were 90.1% of controls on day 43 (2 weeks post-treatment).

  In females: there were decreases in food efficiency (down to 90.4% and 12.3% of controls at 400 and 1000/1250 ppm, respectively) during the first week and to 83.7% of controls at 1000/1250 ppm during the second week. Additionally there were statistically significant decreases in body weight gain during the first 4 days at 400 ppm (down to 72.5% of controls at on day 4) and throughout the experiment at 1000/1250 ppm (e.g. down to 67.8% of controls on day 29). Body weight gains at 400 ppm were comparable to those of controls after the first week of the study. In the female recovery group (1000/1250 ppm), although body weight gains were 63.8% of controls on day 29, they were 89.9% of controls on day 43 (2 weeks post-treatment).

- Statistically significant increases in relative testis weight (up to 116.8% of control) were observed in 1000/1250 ppm males at sacrifice. The recovery group rats (1000/1250 ppm), likewise, showed statistically significantly increases in relative testis weight (up to 121.0% of controls) at sacrifice (2 weeks after the end of treatment). Because there were no histopathological findings in testis, the toxicological significance of the increased relative testicular weights is not clear.

2. Diet preparation

Five diet preparation dates were given (6/4/92, 6/24/92, 7/9/92, 8/7/92, and 8/21/92) corresponding to intervals of about 14 to 29 days. Diets were based on CT1 diet supplied by Special Diet Services Ltd. and stored at -20°C. Aliquots were thawed and presented to the animals daily. Diets were analyzed for stability, homogeneity, and concentration.

Stability studies were conducted with diets stored at room temperature or frozen at -20°C. As shown in Table 2, only 81.7% and 86.5% of the a.i. remained after 3 days at room temperature in diets containing 50 and 1000 ppm, respectively, of the active ingredient. In the case of diets stored initially at -20°C for 21 days, then thawed, and then kept at room temperature for 1 day, 81.9% and 106% of the a.i. was present at 50 and 1000 ppm, respectively. If the diets were stored frozen at -20°C and analyzed (presumably) shortly after thawing, 100.8% and 102.6% of the a.i. was recovered after 39 days of storage at 50 and 1000 ppm, respectively. As noted above, aliquots were thawed and presented to the animals daily.

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Table 2. Stability data for MTI in prepared diets stored at room temperature and at -20°C. (From pp 42-43 of the Study Report, MRID No. 431387-24).

Nominal ppm	Preparation date	Interval (days)	Mean ppm	% of initial ppm
Data obtained at room temperature:				
50	5/11/92	0	51.5	100
		3	42.1	81.7
		4	40.4	78.4
		9	40.4	78.4
	5/11/92[1]	0	52.6	100.0
		1	43.1	81.9
1000	5/11/92	0	1051	100
		3	909	86.5
		4	937	89.2
		9	865	82.3
	5/11/92[1]	0	956	100.0
		1	1016	106.3
Data obtained at -20°C:				
50	5/11/92	0	51.5	100
		21	52.6	102.1
		39	51.9	100.8
1000	5/11/92	0	1051	100
		21	956	91.0
		39	1078	102.6

[1] Diet was prepared on 5/11/92, as indicated, then stored at -20°C until 6/1, at which time the study of stability at room temperature was started.

To analyze for homogeneity, samples were obtained corresponding to the top, middle and bottom of the container for the nominal 50 and 1000 ppm levels for diets prepared on 6/4/92, 6/24/92, 7/9/92, and 8/7/92. As shown in Table 3, blending appears to be homogeneous (means for the various levels are within 10% of the overall mean, except for one diet in which the value is -11.3% of the overall mean).

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Table 3. Analysis of blended diets for homogeneity[1].

Nominal ppm	Mean ppm (% deviation from overall mean)			Overall mean ppm
	Bottom	Middle	Top	
Diet prepared 6/4/92:				
50	49.8 (-6.0)	52.8 (-0.4)	56.4 (+6.4%)	53.0
1000	996 (-0.2)	954 (-4.4)	1044 (+4.6)	998
Diet prepared 6/24/92:				
50	44.0 (-3.3)	48.8 (+7.3)	43.6 (-4.2)	45.5
1000	1152 (+8.8)	1085 (+2.5)	939 (-11.3)	1059
Diet prepared 7/9/92:				
50	50.3 (+3.1)	49.5 (+1.4)	46.5 (-4.7)	48.8
1000	1000 (-2.0)	1047 (+2.6)	1013 (-0.7)	1020
Diet prepared 8/7/92:				
50	59.1 (+5.9)	58.1 (+4.1)	50.3 (-9.9)	55.8
1000	1043 (+4.2)	1009 (+0.8)	950 (-5.1)	1001

[1] Data obtained from pp. 38-41 of the Study Report (MRID No. 431387-24)

To analyze for concentration, all dietary levels were assayed for diets prepared on 6/4/92, 6/24/92, 7/9/92, 8/7/92, and 8/21/92. As shown in Table 4, except for one low value (78% of theoretical) at 50 ppm all analytical concentrations were within 14% or less of the nominal value.

Table 4. Analytical concentration of MTI in test diets[1]

Preparation date	Mean analytical concentration at nominal, ppm (% of theoretical)			
	Control	50	250	1000
6/4/92	ND[2]	47.5 (95.0)	273 (109.2)	1029 (102.9)
6/24/92	ND	43.5 (87.0)	241 (96.4)	1054 (105.4)
7/9/92	ND	48.1 (96.2)	219 (87.6)	998 (99.8)
8/7/92	ND	53.4 (106.8)	249 (99.6)	1063 (106.3)
8/21/92[3]	ND	39.4 (78.8)	215 (86.0)	905 (90.5)

[1] Data obtained from pp. 35-36 of the Study Report (MRID No. 431387-24).
[2] N.D. = Not Detected.
[3] Samples stored in the freezer until analyzed 21/9/92.

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3. The animals were supplied filtered mains water via an automatic water system. Test diet was supplied ad libitum (Basal diet was CT1 diet supplied by Special Services Ltd. Stepfield, Witham, Essex, UK).

4. Statistics

Body weights were analyzed by analysis of covariance on initial body weight. Food consumption, food utilization, hematology, and blood clinical chemistry were analyzed by analysis of variance. Organ weights were considered by analysis of variance and analysis of covariance on final body weight.

5. Compliance:

Statements of data confidentiality (none claimed), adherence to GLPs and Quality Assurance inspections with signatures and dates were included.

C. Methods and Results:

1. Observations:

The animals were observed once daily for behavior and clinical condition. Each rat was examined in detail once a week. There were no deaths during the treatment period. No clinical signs of toxicity attributable to the test material were observed.

2. Body weight:

The animals were weighed immediately before commencement and weekly thereafter through termination. Table 4 shows the group mean body weights and Table 5 shows mean body weight gains during treatment.

As shown in Table 4, statistically significantly decreased body weights were observed throughout the treatment period at 1000 ppm in both sexes (decreased to 84.9% and 86.7% of controls at 14 weeks in males and females, respectively). Additionally, females had statistically significantly decreased body weights throughout treatment at 250 ppm (decreased to 95.6% of controls at 14 weeks).

As shown in Table 5, mean body weight gains by week 14 were decreased to 79.3% of controls in high-dose males. In females, body weight gains decreased in dose-related manner to 89.1% and 68.7% of controls at 250 and 1000 ppm, respectively, at 1-5 weeks. Additionally, body weight gains decreased in dose-related manner to 92.6% and 76.7% of controls at 250 and 1000 ppm, respectively, at 1-14 weeks.

Table 4. Group mean body weights (g/rat) of rats treated with MTI. Data abstracted from pp. 52-55 of the Study Report (MRID 431387-24).

Week	Body weight (adjusted body weight) 0	50	250	1000
Males (n=20/group)				
1	139.6	134.9	133.0	139.0
3	246.5 (241.8)	238.8 (241.5)	235.7 (241.4)	212.4 (208.6)**
5	332.5 (326.4)	311.7 (317.4)	319.8 (325.1)	287.6 (282.5)**
7	389.7 (384.0)	367.1 (370.4)	374.3 (381.4)	338.3 (333.8)**
9	436.0 (430.5)	406.6 (409.8)*	417.0 (423.8)	376.0 (371.5)**
11	470.1 (464.4)	434.9 (438.1)**	447.3 (454.3)	405.0 (400.4)**
14	508.0 (502.3)	470.3 (473.5)**	483.9 (490.8)	431.0 (426.5)**
Females (n=20/group)				
1	119.1	119.6	121.0	121.4
3	172.5 (174.0)	172.6 (173.5)	172.5 (171.5)	155.6 (154.1)**
5	210.9 (212.7)	208.3 (209.3)	202.8 (201.7)**	184.5 (182.8)**
7	233.0 (234.9)	231.5 (232.7)	225.7 (224.4)**	204.2 (202.3)**
9	250.9 (252.4)	246.4 (247.3)	241.1 (240.1)**	220.5 (219.0)**
11	261.8 (263.7)	263.6 (264.7)	254.0 (252.8)**	234.9 (233.0)**
14	276.8 (278.5)	273.9 (274.9)	267.1 (266.1)**	243.0 (241.4)**

* $p \leq 0.05$; ** $p \leq 0.01$.

Table 5. Group mean body weight gain of rats treated with MTI for 90 days[a].

Dose level (ppm)	Group mean body weight gains [in grams] Males 1-5 weeks	Males 1-14 weeks	Females 1-5 weeks	Females 1-14 weeks
0	192.9	368.4	91.8	157.7
50	176.8 (91.7)	335.4 (91.0)	88.7 (96.6)	154.3 (97.8)
250	186.8 (96.8)	350.9 (95.2)	81.8 (89.1)	146.1 (92.6)
1000	148.6 (77.0)	292.0 (79.3)	63.1 (68.7)	121.0 (76.7)

[a] Body weight gains for weeks 1-5 and 1-14 were calculated by the reviewer using the body weight data in Table 4 of this DER. Numbers in parenthesis represent the body weight gain expressed by the reviewer as percent (%) of the body weight gain of control rats.

3. Food consumption and compound intake:

The quantity of food consumed was reported weekly, and is summarized in Table 6. Statistically significant decreases in food consumption vs. controls were found for sexes throughout treatment at the high-dose. Additionally, statistically significant decreases in food consumption throughout treatment were found for low-dose males. The significance of the effect at the low dose is unclear because, there was no decrease in food consumption at the mid-dose.

Food efficiency values (g. growth / 100 g. of food) are summarized in Table 7. Food efficiency values for weeks 1-13 were statistically significantly decreased to 89.6% and 85.4% of controls for high dose males and females, respectively. Food efficiency values for low-dose males during weeks 1-13 although statistically significantly decreased vs controls, were decreased only by 3.2% (i.e. to 96.8% of controls).

No data on water consumption were available for examination.

Table 6. Mean daily food consumption by rats (g/rat/day) treated with MTI. Data abstracted from pp. 56 and 57 of the Study Report (MRID 431387-24).

	Dose level (ppm)			
Week	0	50	250	1000
Males (n=12/group)				
1	24.4	23.2	22.9*	17.6**
3	29.2	27.6**	28.1*	26.1**
5	28.8	27.5	28.0	25.9**
7	29.1	27.1**	28.3	26.5**
9	28.3	26.8*	27.2	25.5**
11	28.4	26.6*	27.1	25.6**
13	27.6	26.0*	26.7	24.3**
Females (n=12/group)				
1	19.2	18.5	18.6	15.1**
3	19.8	20.3	19.5	19.0
5	20.2	19.6	19.4	17.6**
7	19.8	19.9	19.9	17.9**
9	20.1	20.5	19.7	18.3**
11	20.0	19.5	19.2	18.1**
13	18.6	19.0	18.7	16.9**

* $p \leq 0.05$; ** $p \leq 0.01$.

Table 7. Mean food efficiency[1] of rats treated with MTI. Data abstracted from p. 58 of the Study Report (MRID No. 431387-24).

Week	Dose level (ppm) 0	50	250	1000
Males (n=12/group)				
Weeks 1-4	24.99	24.38	25.32	22.57**
Weeks 5-8	12.76	12.02	12.33	12.02
Weeks 9-13	7.24	6.79	6.97	6.21**
Weeks 1-13 (Overall)	14.30	13.84*	14.14	12.82**
Females (n=12/group)				
Weeks 1-4	16.50	16.16	15.08*	12.62**
Weeks 5-8	7.10	6.82	6.91	7.18
Weeks 9-13	3.76	3.92	3.83	3.56
Weeks 1-13 (Overall)	8.72	8.55	8.23	7.45**

[1] Food efficiency: g. of growth / 100 g. of food.
* $p \leq 0.05$; ** $p \leq 0.01$.

Table 8. Mean test material intake (mg/kg/day) for rats treated with MTI for 90 days. Data from pp. 90 and 91 of the Study Report (MRID No. 431387-24).

Dose level (ppm)	Mean test material intake during weeks 1-13 (mg/kg/day) Males	Females
0	0.0	0.0
50	4.1	4.6
250	20.7	23.2
1000	83.0	93.0

4. Ophthalmological examinations:

Ophthalmic observations were done on all control and high-dose animals before treatment and during the week prior to termination. Examination were conducted with a Fison's binocular indirect ophthalmoscope after the pupils had been dilated with 0.5% v/v tropicamide. No treatment-related effects were observed.

5a. Hematology:

Hematology determinations were done on blood obtained by cardiac puncture at termination. The following parameters were determined: Hb, white cell count, red cell count, MCV, MCH, MCHC, hematocrit, platelet count, coagulation

parameters (PT and KCT), and differential white cell count.

In males, there were statistically significant increases vs controls in hemoglobin (by 2.7% at 50 and 250 ppm and by 3.4% at 100 ppm), hematocrit (by 2.7% at 50 and 1000 ppm and by 3.4% at 250 ppm). Additionally, there was a statistically significant 6.1% decrease in platelet counts vs controls at the high dose.

There were no statistically significant changes in hematology parameters in female rats.

Table 9. Summary of selected hematological values[a] in rats treated with MTI. Data abstracted from pp. 59-62 of the Study Report (MRID No. 431387-24).

Parameter	Mean hematological values							
	Males				Females			
	0	50	250	1000	0	50	250	1000
Hemoglobin (g/dl)	14.7	15.1*	15.1*	15.2**	15.0	15.3	15.2	15.2
Hematocrit	0.446	0.458*	0.461**	0.458**	0.447	0.450	0.450	0.449
Red blood cell count ($x10^{12}/l$)	8.50	8.70*	8.74**	8.67	8.17	8.27	8.29	8.24
Platelet count ($x10^{9}/l$)	838	802	822	787*	793	813	803	836

[a] No statistically significant effects on MCV, MCH, MCHC, WBC count and coagulation parameters (PT, KCT) vs controls were found at any dose level or sex. Differential WBC count was done for high-dose rats and controls; no significant differences were observed.

5b. Clinical Chemistry.

Clinical chemistry determinations were done on blood obtained by cardiac puncture at termination. In addition to the parameters listed in Table 10, the following plasma parameters were determined: urea, creatinine, glucose, albumin, cholesterol, total bilirubin, Na^+, K^+, Cl^-, PO_4^{3-}, and gamma-glutamyl transferase.

As shown in Table 10, high-dose males had a statistically significant decrease in triglycerides (decreased by 19.1%) and a statistically significant increase in alkaline phosphatase (increased by 17.8%). Glucose was statistically significantly increased (by 14.2%) at the mid-dose only.

As shown in Table 10, high-dose females had statistically significant decreases in total protein (decreased by 2.6%) and alanine transaminase (decreased by 21.1%).

Table 10 Summary of selected clinical chemistry[a] values in rats treated with MTI. Data abstracted from pp. 63-66 of the Study Report (MRID No. 431387-24).

Parameter	Mean clinical chemistry values							
	Males				Females			
	0	50	250	1000	0	50	250	1000
Plasma glucose (mg/100 ml)	168	168	192*	173	229	215	219	227
Plasma Total protein (g/100 ml)	6.35	6.37	6.47	6.24	6.16	6.24	6.15	6.00*
Plasma triglycerides (mg/100 ml)	115	116	114	93**	63	66	67	63
Plasma alkaline phosphatase (IU/l)	157	171	171	185**	87	99	92	102
Plasma alanine transaminase (IU/l)	52.8	49.7	57.5	58.6	51.7	46.3	44.6	40.8*
Plasma aspartate transaminase (IU/l)	61.9	57.0	63.6	56.2	68.0	59.6*	61.2	58.4*
Plasma creatine kinase (IU/l)	69.3	52.8	80.0	59.3	82.2	49.0**	70.1	76.4

6. Urinalysis.

No urinalysis data were submitted. None are required on a routine basis unless there is an indication based on observed or expected toxicity.

7. Sacrifice and Pathology:

All animals were sacrificed at termination of dosing and were subject to gross pathological examination and the CHECKED (X) tissues listed below were collected for histological examination. The DOUBLE-CHECKED (XX) organs, in addition, were weighed. The collected tissues were examined microscopically from all animals in the control and high-dose (1000 ppm) groups. Macroscopic lesions were observed from all animals.

Digestive System	Cardiovasc./Hemato.	Neurologic
Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Periph. nerve (sciatic)*
X Oral cavity		X Spinal cord[c,e]
X Esophagus*	X Bone marrow*	X Eyes[c]
X Stomach*	X Lymph nodes*	
X Duodenum*	X Spleen*	Glandular
X Jejunum*	X Thymus*	XX Adrenal gland*
X Ileum*	Urogenital	Lacrymal gland[c]
X Cecum*	XX Kidneys*[a]	X Mammary gland[c]
X Colon*	X Urinary bladder*	X Parathyroid*
X Rectum*	XX Testes*[a]	X Pituitary*[d]
	X Seminal vesicles	X Thyroid*[d]
XX Liver*[a]	X Epididymides	Other
Gall bladder[b]	X Prostate	X Bone (with b. marrow)[c]
X Pancreas*	X Uterus*	X Voluntary muscle[c]
Respiratory	X Ovaries	X Skin
X Trachea[c]	Oviduct	X Any abnormal tissue
X Lungs*	Vagina	X Harderian glands
X Nasal passages		X Sternum

* Required for subchronic oral studies (EPA Guideline 82-1).
[a] Organ weights required in subchronic rodent oral studies (EPA Guideline 82-1).
[b] Not present in rats.
[c] Required only if indicated by signs of toxicity or target organ involvement.
[d] Parathyroids were removed together with the thyroid.
[e] Number of levels examined was not indicated (three levels necessary when required).

a. Organ weights

A summary of mean male/female absolute (g), relative organ weights (% b.w.), and covariance-adjusted body weights (g) for the final sacrifice period is presented below in Tables 11 (males) and 12 (females).

In males, there were statistically significant decreases vs controls in absolute weights for adrenals (-6.6%), brain (-3.9%), kidneys (-17.4.0%), and liver (-14.8%) at the high dose. Relative organ weights, however, were not statistically significantly decreased at any dose level. There were no statistically significant effects on absolute or relative testicular weights.

In females, there were statistically significant decreases vs controls in absolute weights for adrenals (-10.3%), kidneys (-11.2%), and liver (-12.7%) at the high dose. Relative organ weights, however, were not statistically significantly decreased at any dose level.

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Table 11. Summary of Absolute (g), Organ-to-Body weight (% b.w.), and adjusted (g) values in male rats treated with MTI. Data from pp. 67-71 of the Study Report (MRID No. 431387-24).

	Mean Absolute (g), Relative[1] (%) and Adjusted[2] (g) values			
Organ	0	50	250	1000
ADRENAL GLANDS				
Terminal body weight[3]:	508.0	477.7[4]	483.9	431.0
Absolute:	0.061	0.060	0.056*	0.057*
Relative:	0.012	0.013	0.012	0.013
Adjusted:	0.060	0.060	0.055	0.059
BRAIN:				
Terminal body weight:	508.0	470.3	483.9	431.0
Absolute:	2.05	2.02	2.02	1.97**
Relative:	0.40	0.43	0.42	0.46
Adjusted:	2.01	2.02	2.00	2.02
KIDNEYS:				
Terminal body weight:	508.0	473.1[5]	486.7[5]	431.0
Absolute:	3.40	3.05**	3.07**	2.81**
Relative:	0.67	0.65	0.63	0.65
Adjusted:	3.16	3.07	2.99*	3.11
LIVER:				
Terminal body weight:	508.0	473.1	483.9	431.0
Absolute:	20.9	18.9**	19.8	17.8**
Relative:	4.1	4.0	4.1	4.1
Adjusted:	19.1	19.0	19.3	20.1
TESTES:				
Terminal body weight:	508.0	470.3	480.2	431.0
Absolute:	3.48	3.45	3.38	3.54
Relative:	0.69	0.74	0.71	0.82
Adjusted:	3.43	3.45	3.37	3.59*

[1] Relative = (absolute organ weight x 100) / (terminal body weight).
[2] Adjusted by covariance on body weight.
[3] N=20, unless specified to be 18 or 19.
[4] N=18.
[5] N=19.

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Table 12. Summary of Absolute (g), Organ-to-Body weight (% b.w.), and adjusted (g) values in female rats treated with MTI. Data from pp. 67-71 of the Study Report (MRID No. 431387-24).

	Mean Absolute (g), Relative[1] (%) and Adjusted[2] (g) values			
Organ	0	50	250	1000
ADRENAL GLANDS				
Terminal body weight:	276.8	273.9	267.1	243.0
Absolute:	0.078	0.078	0.076	0.070[3]
Relative:	0.028	0.029	0.028	0.029
Adjusted:	0.077	0.077	0.076	0.072
BRAIN:				
Terminal body weight:	276.9	273.9	267.1	243.0
Absolute:	1.86	1.86	1.87	1.83
Relative:	0.67	0.68	0.70	0.76
Adjusted:	1.84	1.85	1.87	1.86
KIDNEYS:				
Terminal body weight:	276.8	273.9	267.1	243.0
Absolute:	1.88	1.89	1.85	1.67[3]
Relative:	0.68	0.69	0.69	0.69
Adjusted:	1.83	1.86	1.84	1.77
LIVER:				
Terminal body weight:	276.8	273.9	267.1	242.1[5]
Absolute:	10.2	10.1	9.9	8.9[3]
Relative:	3.7	3.7	3.7	3.7
Adjusted:	9.9	9.9	9.8	9.6

[1] Relative = (absolute organ weight x 100) / (terminal body weight).
[2] Adjusted by covariance on body weight.
[3] N=20, unless specified to be 18 or 19.
[4] N=18.
[5] N=19.

b. Gross pathology

Gross pathology examination of the animals at terminal sacrifice did not reveal any any treatment-related effects. Gross pathology observations included:

In Males: A dilated pelvis in 6/20, 3/20, 5/20, and 2/20 rats at 0, 50, 250, and 1000 ppm, respectively, and 1/20 rats had flaccid testis at the mid-dose.

In Females: A dilated pelvis in 1/20, 1/20, 3/20, and 2/20 rats at 0, 50, 250, and 1000 ppm, respectively.

c. Microscopic pathology

Microscopic pathology findings at terminal sacrifice for controls and high-dose

rats are shown in Attachment 1. As summarized in Attachment 1, there were no apparent treatment-related histological findings. There no statistically significant differences between controls and high-dose rats of either sex in the incidence of microscopic changes.

It is noted, however, that 1/20 high-dose males (rat No. 74) vs 0/20 controls showed increased hemosiderin (minimal) in the spleen. This effect appears to be incidental, as the hematology data are not consistent with an increased destruction rate of red blood cells. It was not possible to examine the hematology parameters of rat 74, because the hematology data for this rat were not included with the individual animal data.

In the case of females, 1/20 high-dose rats vs 0/20 controls showed sciatic nerve fiber degeneration (minimal). This effect appears to be incidental because in addition to its minimal incidence in high-dose females, it was also observed in 3/20 control males.

D. Discussion:

MTI was administered to Alpk:APfSD (Wistar derived) rats of both sexes for a period of 90 days in the diet at dose levels of 0, 50, 250, or 1000 ppm, corresponding to mean MTI intake of 0, 4.1, 20.7 or 83 mg/kg/day (in males) and 0, 4.6, 23.2, or 93.0 mg/kg/day (in females).

Stability studies at room temperature indicated that up to 18% of the active ingredient could disappear in 1 day at the low dose. At the high dose, however, the diet appeared to be fully stable during a 24-hour period. Values for homogeneity and concentration of MTI in the diet were found to be adequate.

Statistically significantly decreased body weights were observed at 1000 ppm in males throughout the treatment period (e.g. to 84.8% of controls at 14 weeks). Mean body weight gains at 250 and 1000 ppm in males were reduced to 95.2 and 79.3% of controls for weeks 1-14. Mean food efficiency values for high dose males were statistically significantly decreased for weeks 1-13 (to 89.7% of controls), 1-4 (to 90.3% of controls), and 9-13 (to 85.8% of controls). It is noted that although there were no statistically significant differences in body weight between 250 ppm males and controls, 50 ppm males had statistically significantly decreased body weights (to 92.6% of controls) during the latter of the treatment period, in addition to a small (to 96,8% of controls) but statistically significant decrease in mean food efficiency for the 1-13 week period.

Statistically significantly decreased body weights were observed at 250 and 1000 ppm in females throughout the treatment period (e.g. to 94.5 and 87.8% of controls at 14 weeks for 250 and 1000 ppm, respectively). Likewise, mean body weight gains decreased in dose-related fashion to 89.1% and 68.7% of controls at 250 and 1000 ppm, respectively, at 1-5 weeks and to 92.6% and 76.7% of controls, at 250 and 1000 ppm, respectively, for weeks 1-14. Mean food efficiency values for weeks 1-4 decreased with increasing dose, reaching statistical significance at 250 and 1000 ppm, at 91.3 and 76.5% of controls, respectively.

Changes in hematology and clinical chemistry parameters did not follow a clear

dose-related trend and appeared to be incidental to the treatment.

Although there were statistically significant decreases in absolute organ weights for adrenals, brain, kidneys, and liver in high-dose males, there were no apparent effects on relative or covariance-adjusted weights for these organs. There were statistically significant increases (to 104.7% of controls) in covariance-adjusted testes weights in high-dose rats, coupled to increases (to 118.8% of controls) in relative testes weights that were not statistically significant. It is noted that statistically significant increases in testes weights (to 116.8% of controls) were observed at 1250/1000 ppm in the 28-day range finding study. These increases in testes weights appear to be of uncertain toxicological significance in the absence of histological effects on testes.

There were no apparent treatment-related histological findings.

This study defines a LEL of 250 ppm (♂: 20.7 mg/kg/day; ♀: 23.2 mg/kg/day) based on dose-related and statistically significant, decreases in food efficiency for females (down to 91.3 and 76.5% of controls at 250 and 1000 ppm for weeks 1-4), coupled to dose-related decreases in body weight gain (down to 89.1 and 68.7% of controls at 250 and 1000 ppm for weeks 1-4). The NOEL is 50 ppm (♂: 4.1 mg/kg/day; ♀: 4.6 mg/kg/day).

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011613

Reviewed by: Alberto Protzel, Ph.D.
Review Section III, Toxicology Branch II(7509C) 4/19/95
Secondary Review by: James N. Rowe, Ph.D.
Review Section III, Toxicology Branch II(7509C) 4/20/95

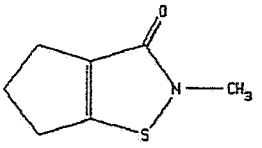
DATA EVALUATION RECORD

STUDY TYPE: Subchronic oral
Species: Beagle Dog
EPA Guideline 82-1

EPA IDENTIFICATION: EPA MRID No. 431387-26
PC Code: 107107
DP Barcode: D208776 and D210001
Case: 040889
Submission No: S475970
EPA ID#: 010182-GIL

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS/STRUCTURE: MTI



STUDY NUMBER: PD0931 (Report No. CTL/P/3972).

SPONSOR: Zeneca Inc. Zeneca Specialties. Wilmington, DE 19897.

TESTING FACILITY: Zeneca Central Toxicology Laboratory. Alderley Park, Macclesfield, Cheshire, UK.

TITLE OF REPORT: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): 90 Day Dietary Study in Dogs.

AUTHOR: N. J. Rattray

REPORT ISSUED: July 28 1993.

EXECUTIVE SUMMARY:

In a 13-week subchronic feeding study, MTI was administered to beagle dogs of both sexes for a period of 90 days in the diet at dose levels of 0, 100, 300, or 1000 ppm, corresponding to mean MTI intake of 0, 3.1, 9.3 or 31.5 mg/kg/day (in males) and 0, 3.2, 10.1, or 33.4 mg/kg/day (in females). There were no effects on body weights or body weight gains for both sexes. These effects are in contrast with those observed in the pilot study [MRID 431387-25]: body weight gains in females at 928 and 464 ppm in the pilot study were 36.4% and 54.5% of those of controls, respectively. Changes in hematology and clinical chemistry parameters did not follow a clear dose-related trend and appeared to be incidental to the treatment. No clear-cut, dose-related effects were observed

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upon macroscopic examination of the dogs. Although clear macroscopic signs of irritation of the tongue were seen in 1392 and 928 ppm females in the pilot study, no signs of tongue irritation were seen in this study. There were no apparent treatment-related histological findings. This study does not define a LOEL due to the absence of clear-cut, dose-related effects. The NOEL is 1000 ppm, the highest dose tested (♂: 31.5 mg/kg/day; ♀: 33.4 mg/kg/day).

This study is classified as Core Minimum. This study satisfies the requirement, § 82-1 for a subchronic oral toxicity in non-rodents.

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o Effects on body weights: At 1392 ppm, body weights decreased by 9.5% and 0.9% during the first week in males and females, respectively. Food consumption at 1392 ppm, had also decreased to 50 and 84% of controls during the first week, in males and females, respectively. The high-dose dogs were sacrificed after one week. At 928 ppm, although the dogs ate slowly, food intake was comparable to controls. Body weight gain in males was comparable to controls. Body weight gains in females at 928 and 464 ppm were 36.4% and 54.5% of those of controls, respectively.

o Macroscopic pathology findings: At 1392 ppm the female had a dark red area on the tongue (10% of tongue) and reddening of the urinary bladder neck. At 928 ppm, the female had a red area on the gastroduodenal mucosa in the stomach (this animal had 25% of her tongue reddened on day 7 with return to normal by day 21).

o Microscopic pathology findings: At 1392 ppm both dogs showed minimal regeneration of surface epithelium in the duodenum and there were minor inflammatory changes in the jejunum of the female. The appearance was characteristic of regeneration following injury. The tongue of the female showed epithelial necrosis with microabcessation, acanthosis and parakeratosis, effects that are consistent with irritation. The epididymis of the male dog showed slight tubular necrosis/sperm granulomata and moderate vacuolation/degeneration of the tubular epithelium; these findings in the epididymis were not considered to be treatment-related. At 928 ppm no histological changes were found to account for the reddening of the gastroduodenal mucosa observed macroscopically at this dose level. No histopathology was observed at 464 ppm.

The LOEL was considered to be 464 ppm, based on the decreased body weight-gain of the female at this dose level. Based on the above results of the pilot study, dietary levels of MTI were recommended as 100, 300 and 900 ppm for the subchronic toxicity study in dogs.

2. Diet preparation

Three diet preparation dates were given [10/15/92 (about 1 week before the start of dosing), 11/27/92, and 1/4/93) corresponding to intervals of about 6 weeks. Diets were based on powdered laboratory diet A supplied by Special Diet Services Ltd. Stepfield, Witham, Essex, UK and stored at -20°C. Aliquots were thawed and presented to the animals daily. Diets were analyzed for stability, homogeneity, and concentration.

Stability studies were conducted with diets stored at room temperature or frozen at -20°C. As shown in Table 2, 90.4% and 89.5% of the a.i. remained after 1 day at room temperature in diets containing 50 and 1000 ppm, respectively, of the active ingredient. The value of 89.5%, may actually be a lower estimate, since at 2 days the residual a.i. amounted to 91.2%. If the diets were stored frozen at -20°C and analyzed (presumably) shortly after thawing, 116.1% and 94.0% of the a.i. was recovered at 100 and 1000 ppm, respectively, after 45 days of storage. In another experiment, 88.6% and 96.4% of the a.i. was recovered at 100 and 1000 ppm after 41 days of storage at -21°C. As noted above, aliquots were thawed and presented to the animals daily.

Table 2. Stability data for MTI in prepared diets stored at room temperature and at -20°C. (From pp. 30 and 31 of the Study Report, MRID No. 431387-26).

Nominal ppm	Preparation date	Interval (days)	Mean ppm	% of initial ppm
Data obtained at room temperature:				
100	10/15/92	0	92.8	100
		1	83.9	90.4
		2	76.0	81.9
1000	10/15/92	0	1065	100
		1	953	89.5
		2	971	91.2
Data obtained at -20°C:				
100	10/15/92	0	92.8	100.0
		45	107.7	116.1
		79	74.5	80.3
		86	66.1	71.2
1000	10/15/92	0	1065	100.0
		45	1001	94.0
		79	900	84.5
		86	885	83.1
100	11/27/92	0	97.1	100.0
		41	86.0	88.6
1000	11/27/92	0	941	100.0
		41	907	96.4

To analyze for homogeneity, samples were obtained corresponding to the top, middle and bottom of the container for the nominal 100 and 1000 ppm levels for a test diet prepared on 10/15/92. As shown in Table 3, blending appears to be homogeneous (means for the various levels are within 3% of the overall mean.

Table 3. Analysis of blended diets for homogeneity[1].

Nominal ppm[2]	Mean ppm (% deviation from overall mean)			Overall mean ppm
	Bottom	Middle	Top	
100	93.5 (+1.1)	94.1 (+1.7)	89.9 (-2.8)	92.5
1000	1000 (-1.8%)	1037 (+1.9)	1018 (0.0)	1018

[1] Data obtained from p. 29 of the Study Report (MRID No. 431387-26).
[2] Homogeneity studies were done with a test diet prepared 10/15/92, approximately 1 week before the initiation of dosing.

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To analyze for concentration, all dietary levels were assayed for diets prepared on 10/15/92 (approximately 1 week prior to the start of treatment), 11/27/92, and 1/4/93. As shown in Table 4, all mean analytical concentrations were within 7.2% or less of the nominal value.

Table 4. Analytical concentration of MTI in test diets[1]

Preparation date	Mean analytical concentration at nominal, ppm (% of theoretical)			
	Control	100	300	1000
10/15/92	ND[2]	92.8 (92.8%)	305 (101.7%)	1065 (106.5%)
11/27/92	ND	97.1 (97.1%)	313 (104.3%)	941 (94.1%)
1/4/93	ND	97.3 (97.3%)	315 (105.0%)	1003 (100.3%)

[1] Data obtained from p. 28 of the Study Report (MRID No. 431387-26).
[2] ND = Not Detected.

3. The animals were supplied filtered mains water via an automatic water system. Test diet was supplied ad libitum (Basal diet was CT1 diet supplied by Special Services Ltd. Stepfield, Witham, Essex, UK).

4. Statistics

Body weights were analyzed by analysis of covariance on initial body weight. Hematology, and blood clinical chemistry were analyzed by analysis of covariance on pre-experimental values. Organ weights were considered by analysis of variance and analysis of covariance on final body weight.

5. Compliance:

Statements of data confidentiality (none claimed), adherence to GLPs and Quality Assurance inspections with signatures and dates were included.

C. Methods and Results:

1. Observations:

The dogs were observed at least twice daily for behavior and clinical condition. Stool consistency was examined daily. Each dog was examined in detail once a week; all dogs were also given a full clinical examination by a veterinarian on week -1 and prior to termination. The examination included cardiac and pulmonary auscultation and indirect ophthalmoscopy. There were no deaths during the treatment period. No clinical signs of toxicity attributable to the test material were observed.

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2. Body weight:

All dogs were weighed weekly, before feeding, throughout the pre-experimental period, on day 1 and thereafter at weekly intervals during treatment. Table 4 shows the group mean body weights and Table 5 shows mean body weight gains during treatment.

As summarized in Table 4, there were no treatment-related effects on mean body weights. A statistically significant decrease in mean body weights was observed in 300 ppm females, this effect is probably incidental due to the absence of dose-relatedness.

As shown in Table 5, mean body weight gains by week 14 were increased to 111-119% of controls in all male dose groups. In females, except for mid-dose animals, body weight gains were within 6% of controls.

Table 4. Group mean body weights (kg/dog) of dogs treated with MTI. Data abstracted from pp. 43 & 44 of the Study Report (MRID 431387-26).

	Body weight [in kg/dog]			
Week	0	100	300	1000
Males (n=4/group)				
1	11.65	11.73	11.45	11.38
3	12.35	12.48	12.13	12.10
5	12.65	12.85	12.58	12.53
7	13.10	13.23	13.03	12.93
9	13.30	13.63	13.38	13.25
11	13.58	13.90	13.75	13.60
14	13.83	14.15	14.05	13.93
Females (n=4/group)				
1	9.35	9.90	9.48	9.35
3	9.85	10.43	9.90	9.98
5	10.23	10.85	10.18	10.25
7	10.65	11.18	10.43*	10.68
8	10.88	11.40	10.60**	10.80
9	11.05	11.48	10.75	10.93
11	11.28	11.75	11.00	11.20
14	11.55	12.03	11.15	11.43

* $p \leq 0.05$; ** $p \leq 0.01$.

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Table 5. Group mean body weight gain of dogs treated with MTI for 90 days[1].

Dose level (ppm)	Group mean body weight gains [in Kg.] for weeks 1-14	
	Males	Females
0	2.18	2.20
100	2.42 (111.0%)	2.13 (96.8%)
300	2.60 (119.3%)	1.67 (75.9%)
1000	2.55 (117.0%)	2.08 (94.5%)

[1] Body weight gains for weeks 1-14 were calculated by the reviewer using the body weight data in Table 4 of this DER. Numbers in parenthesis represent the body weight gain expressed by the reviewer as percent (%) of the body weight gain of control dogs.

3. Food consumption and compound intake:

Food residues were recorded daily prior to giving the next meal. Measurements were made for at least 2 weeks prior to the experiment and throughout the treatment period. There was no residual food during the treatment period: the animals ate all the food that they were offered during the treatment period. No data on water consumption were available for examination.

Table 8. Mean test material intake (mg/kg/day) for dogs treated with MTI for 90 days. Data from pp. 104 and 105 of the Study Report (MRID No. 431387-26).

Dose level (ppm)	Mean test material intake during weeks 1-13 (mg/kg/day)	
	Males	Females
0	0.0	0.0
100	3.1	3.2
300	9.3	10.1
1000	31.5	33.4

4. Ophthalmological examinations:

All dogs were given a full clinical examination by a veterinarian in week -1 and prior to termination. The examination included indirect ophthalmoscopy. No treatment-related effects were observed.

5a. Hematology:

Hematology determinations were done on blood obtained from the jugular vein of all dogs before feeding, on weeks -1, 4, 8, and 13. The following parameters were determined: Hb, hematocrit, RBC count, MCV, MCH, MCHC, total WBC count, platelet count, coagulation parameters (PT and KCT), and differential white cell count. In males, there were statistically significant increases vs controls in

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adjusted mean MCV (by 2.4%), and MCH (by 3.6%) at 1000 ppm. In females, there were statistically significant increases in adjusted mean prothrombin time (by 6.8%) at 1000 ppm.

5b. Clinical Chemistry.

Clinical chemistry determinations were done on blood obtained from the jugular vein in weeks -1, 4, 8, and 13. The following parameters were determined in plasma: alanine transaminase, aspartate transaminase, creatine kinase, alkaline phosphatase and gamma-glutamyl transferase activities, urea, creatinine, glucose, albumin, plasma triglycerides, total protein, cholesterol, total bilirubin, Ca^{++}, Na^{+}, K^{+}, Cl^{-}, PO_4^{3-}.

There were no consistent and systematic changes in clinical chemistry parameters. Males showed a statistically significant increase in mean gamma-glutamyl transferase (2.2 times the control value) on week 8. High-dose females had a statistically significant increase in adjusted mean cholesterol (increased by 18.4% vs controls at week 13).

6. Urinalysis.

No urinalysis data were submitted. None are required on a routine basis unless there is an indication based on observed or expected toxicity.

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7. Sacrifice and Pathology:

All animals were sacrificed at termination of dosing and were subject to gross pathological examination and the CHECKED (X) tissues listed below were collected for histological examination. The DOUBLE-CHECKED (XX) organs, in addition, were weighed. The collected tissues were examined microscopically from all animals. Macroscopic lesions were observed from all animals.

Digestive System	Cardiovasc./Hemato.	Neurologic
X Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Periph. nerve (sciatic)*
X Oral cavity		X Spinal cord[c,d]
X Esophagus*	X Bone marrow*	X Eyes[c]
X Stomach*	X Lymph nodes*	
X Duodenum*	X Spleen*	Glandular
X Jejunum*	X Thymus*	XX Adrenal gland*
X Ileum*	Urogenital	Lachrymal gland[c]
X Cecum*	XX Kidneys*[a]	X Mammary gland[c]
X Colon*	X Urinary bladder*	X Parathyroid*
X Rectum*	XX Testes*[a]	X Pituitary*
	Seminal vesicles	X Thyroid*
XX Liver*[a]	XX Epididymides	Other
X Gall bladder[b]	X Prostate	X Bone
X Pancreas*	XX Uterus*	X Voluntary muscle[c]
Respiratory	X Ovaries	X Skin
X Trachea[c]	Oviduct	X Any abnormal tissue
X Lungs*	Vagina	Harderian glands
Nasal passages		Sternum

* Required for subchronic oral studies (EPA Guideline 82-1).
[a] Organ weights required in subchronic rodent oral studies (EPA Guideline 82-1).
[b] Not present in rats.
[c] Required only if indicated by signs of toxicity or target organ involvement.
[d] Number of levels examined was not indicated (three levels necessary when required).

a. Organ weights

As summarized below in Tables 11 (males) and 12 (females) there were no treatment-related effects on mean absolute (g) or relative organ weights (% b.w.) in either sex for the final sacrifice period.

Table 11. Summary of Absolute (g) and Organ-to-Body weight (% b.w.) in male dogs treated with MTI. Data from pp. 83-89 of the Study Report (MRID No. 431387-26).

	Mean Absolute (g) and Relative[1] (%) values			
Organ	0	100	300	1000
Terminal body weight	13825	14150	14050	13925
ADRENAL GLANDS				
Absolute:	1.17	1.29	1.22	1.09
Relative:	0.01	0.01	0.01	0.01
BRAIN:				
Absolute:	79.0	83.4	84.0	83.8
Relative:	0.58	0.59	0.60	0.61
EPIDIDYMIDES				
Absolute:	3.47	3.90	3.60	3.39
Relative:	0.02	0.03	0.03	0.02
KIDNEYS:				
Absolute:	56.2	58.3	58.5	57.0
Relative:	0.41	0.41	0.42	0.41
LIVER:				
Absolute:	434	435	414	416
Relative:	3.17	3.09	2.95	3.01
TESTES:				
Absolute:	20.5	22.6	21.4	21.3
Relative:	0.15	0.16	0.15	0.16
THYROID:				
Absolute:	1.11	1.24	1.05	1.16
Relative:	0.008	0.009	0.007	0.008

[1] Relative = (absolute organ weight x 100) / (terminal body weight).

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Table 12. Summary of Absolute (g) and Organ-to-Body weight (% b.w.) in female dogs treated with MTI. Data from pp. 83-89 of the Study Report (MRID No. 431387-26).

	Mean Absolute (g) and Relative[1] (%) values			
Organ	0	100	300	1000
Terminal body weight	11550	12025	11150	11425
ADRENAL GLANDS				
Absolute:	1.18	1.39*	1.20*	1.28
Relative:	0.01	0.01	0.01	0.01
BRAIN:				
Absolute:	74.9	74.9	76.8	72.6
Relative:	0.65	0.63	0.69	0.64
KIDNEYS:				
Absolute:	48.7	49.9	49.2	50.2
Relative:	0.42	0.42	0.44	0.44
LIVER:				
Absolute:	366	384	365	348
Relative:	3.19	3.20	3.29	3.05
THYROID:				
Absolute:	0.80	0.93*	0.95*	0.81
Relative:	0.007	0.008	0.009	0.007

[1] Relative = (absolute organ weight x 100) / (terminal body weight).
* Statistically significantly different from controls ($p<0.05$).

b. Gross pathology

Selected macroscopic observations are summarized in Table 13. Gross pathology observations included:

In Males: Red spots or areas in the esophagus in 1/4 dogs (dog #325) at 1000 ppm and 0/4 in all other groups; red areas in the lung in 1/4 dogs (#327) at 1000 ppm and 0/4 in all other groups.

In Females: Discolored areas in the kidney in 1/4 dogs (dog #329) at 1000 ppm and 0/4 in all other groups; red spots in the stomach in 1/4 dogs (dog #332) at 1000 ppm and 0/4 in all other groups. Additionally, there was reddening of the urinary bladder mucosa 1/4 dogs at 300 and 1000 ppm, and 0/4 dogs at 0, and 100 ppm.

In contrast with the pilot experiment, reddening of the tongue was not reported, even though the tongue was examined in this study.

Table 13. Selected macroscopic observations in dogs treated with MTI. Data from pp. 90-92 of the Study Report (MRID No. 431387-26).

	Incidence of macroscopic observations at terminal sacrifice							
	Males				Females			
Finding	0	100	300	1000	0	100	300	1000
Number examined[1]	4	4	4	4	4	4	4	4
Duodenum								
Red area/s mucosa	1	1	1	1	2	1	1	0
Discolored areas	1	1	0	0	0	1	0	0
Esophagus								
Red spot/s	0	0	0	1	0	0	0	0
Ileum								
Red area/s mucosa	0	1	0	1	0	1	0	0
Jejunum								
Red area/s mucosa	0	1	0	0	0	0	0	0
Kidney								
Discolored area	0	0	0	0	0	0	0	1
Lung								
Pale area/s	1	0	2	0	0	0	0	0
Red areas	0	0	0	1	0	0	0	0
Red spots	0	0	0	0	1	0	0	0
Lymph node pre-scapular								
Discolored	0	0	0	1	2	0	0	1
Stomach								
Red spot/s	1	0	1	0	0	0	0	1
Urinary Bladder								
Red area/s mucosa	0	1	1	0	2	2	0	1
Red spot/s mucosa	0	0	0	1	0	2	3	2
Mucosa reddened	0	0	0	0	0	0	1	1

[1] All observations were conducted in 4 dogs/dose level/sex.

c. Microscopic pathology

Microscopic pathology findings at terminal sacrifice are shown in Attachment 1. As summarized in Attachment 1, there were no apparent treatment-related histological findings. As summarized in Table 14 (data from Attachment 1), 4/4 male high-dose dogs had pneumonitis vs 2/4 controls and 1/4 high-dose dogs had a hemorrhagic lymphoid nodule vs 0/4 in other groups. Additionally, 2/4 high-dose females had histiocytosis in a pre-scapular lymph node vs 0/4 in other groups.

One of 4 dogs at the high-dose, in both sexes, had a segmental inflammation of the spinal artery vs 0/4 in other groups.

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Table 14. Selected microscopic observations in dogs treated with MTI. Data from pp. 93-96 of the Study Report (MRID No. 431387-26).

	Incidence of microscopic observations at terminal sacrifice							
	Males				Females			
Finding	0	100	300	1000	0	100	300	1000
Esophagus								
Number examined	4	4	4	4	4	4	4	4
Lymphoid nodule - (hemorrhagic)	0	0	0	1	0	0	0	0
Lung								
Number examined	4	4	4	4	4	4	4	4
Pneumonitis	2	1	3	4	2	1	1	0
Lymph node (pre scapular)								
Number examined	4	4	4	4	4	4	4	4
Sinus histiocytosis	1	0	1	0	0	0	0	2
Spinal Cord								
Number examined	4	4	4	4	4	4	4	4
Segmental inflammation of spinal artery	0	0	0	1	0	0	0	1

D. Discussion:

MTI was administered to beagle dogs of both sexes for a period of 90 days in the diet at dose levels of 0, 100, 300, or 1000 ppm, corresponding to mean MTI intake of 0, 3.1, 9.3 or 31.5 mg/kg/day (in males) and 0, 3.2, 10.1, or 33.4 mg/kg/day (in females).

Stability studies at room temperature indicated that 90.4% and 89.5% of the a.i. remained after 1 day at room temperature in diets containing 50 and 1000 ppm, respectively, of the active ingredient. Diets were thawed and presented to the animals daily. Values for homogeneity and concentration of MTI in the diet were found to be adequate.

There were no effects on body weights or body weight gains for both sexes. These effects are in contrast with those observed in the pilot study: body weight gains in females at 928 and 464 ppm in the pilot study were 36.4% and 54.5% of those of controls, respectively.

Changes in hematology and clinical chemistry parameters did not follow a clear dose-related trend and appeared to be incidental to the treatment.

No clear-cut, dose-related effects were observed upon macroscopic examination of the dogs. Although clear macroscopic signs of irritation of the tongue were seen in 1392 and 928 ppm females in the pilot study (pp. 3-4 of this DER), no signs of tongue irritation were seen in this study. Although red areas or spots were observed in tissues of some high-dose animals in this study, these observations were confounded by the finding of mucosal red spots/areas at lower doses also.

Reddening of the mucosa of the urinary bladder was seen in 1/4 females at 300 (dog #324) and 1000 (dog #332) ppm; microscopic observations, however, indicated mucosal congestion/hemorrhage in 1/4 dogs at 100 (dog #314) and 1000 (dog #330) ppm.

There were no apparent treatment-related histological findings. It is unclear at this time if the segmental inflammation of the spinal artery seen in 1/4 high-dose is a treatment related effect.

As submitted, this study does not define a LOEL due to the absence of clear-cut, dose-related effects. The NOEL is 1000 ppm, the highest dose tested (♂: 31.5 mg/kg/day; ♀: 33.4 mg/kg/day). Although no clear toxic effect was observed in this study at 1000 ppm, it is clear from the results of the pilot study [MRID 431387-25] that frank macroscopic and microscopic effects are seen at 1392 ppm. There is some concern, however, that this study does not show the some of the effects observed in the pilot study [MRID 431387-25], at equal or lower doses (e.g. tongue irritation in females at 928 ppm, or decreased body weight gains in females at 928 and 464 ppm).

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Attachment 1.
Summary of Microscopic Findings.
From pages 93-96 of the Study Report [MRID No. 431387-26].

EPA Reg. No. 10182-385

Page_____ is not included in this copy.

Pages 107 through 110 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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GUIDELINE: 83-3

Primary Review by: James N. Rowe, Ph.D. James N. Rowe 12/28/94
Review Section 3, Toxicology Branch II/HED

Secondary Review by: Susan Makris, M.S. Susan J Makris 12/29/94
Review Section 3, Toxicology Branch II/HED

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Wistar-derived rats (Alpk:APfsD)(SPF)
Guideline: 83-3(a)

EPA Identification Nos.: DP Barcode No. D210001/D208776
EPA MRID No. 431387-27
EPA Pesticide Chemical Code No. 107107
Caswell No. (not assigned)
EPA ID # 010182-GIL
Cas No.

Test Material: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)

Synonyms: Promexal X50 Preservative

Sponsor: ZENECA Inc.
ZENECA Specialties
Wilmington, Delaware 19897

Study Number(s): CTL/P/3921; No. RR0603

Testing Facility: ZENECA Central Toxicology Laboratory
Alderley Park, Macclesfield,
Cheshire UK

Title of Report: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI): developmental toxicity study in rats

Author(s): P.J. Pinto

Report Issued: March 29, 1993.

Executive Summary:

This study was designed to assess the developmental toxicity potential of MTI in mated female rats (groups of 24/dose) gavaged during gd 7-16 (inclusive) at dose levels of 0, 5, 15 and 40 mg/kg/day.

Maternal toxicity was established for the HDT by 1) the observation of 2 deaths observed on gds 12 and 13 (1 in extremis, 1 found dead), 2) clinical signs of toxicity in 4 dams (including the two which died) related to the respiratory system (abnormal noise, labored breathing, gasping, reduced breathing rate), 3) statistically significant depressions in mean body weights (gds 8-16 inclusive) of 4-6.5% which remained decreased

following cessation of dosing, 4) a 62% decrement in mean body weight gain during the dosing period, and 5) statistically significant depressions in mean food consumption on gds 7-10, 10-13 and 13-16.

There were no apparent dose-related effects upon mean implantations/dam, live fetuses/dam, resorptions/dam (early, late), postimplantation losses, the sex ratios, mean fetal weights or external/visceral/skeletal anomalies.

Maternal toxicity NOEL = 15 mg/kg/day; LOEL = 40 mg/kg/day (HDT). This is based upon increased mortality and clinical signs (respiratory system), depressed mean body weight and weight gain and decreased food consumption.

Developmental NOEL = 40 mg/kg/day (HDT); LOEL = not determined.

Core Classification: Minimum; this study meets the requirements for a developmental toxicity study in rats [§83-3(a)].

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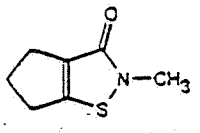
A. Materials

A copy of the "materials and methods" section from the investigators report is appended (pgs. 12-19).

Test Compound: Purity: 94.6%
Description: light brown powder
Lot No.: NBY 2257/69-MTI/SOL
Contaminant: see Certificate of Analysis



Vehicle(s): Deionized water (CTL/Y04517/015)

Test Animal(s): Species: rat
Strain: Wistar derived (Alpk:APfSD)(SPF)
Source: Barriered Animal Breeding Unit, Biologial Services Section, Alderley Park, Macclesfield, Cheshire, UK
Age: approximately 11 weeks old, virgin females
Weight: on arrival, weights ranged from 216-293g

B. Study Design

This study was designed to assess the developmental toxicity potential of MTI in mated female rats (groups of 24/dose) gavaged during gd 7-16 (inclusive) at dose levels of 0, 5, 15 and 40 mg/kg/day.

Mating

Virgin female rats were paired overnight with unrelated males of the same strain in the breeding facility. On the following morning, vaginal smears were examined for the presence of sperm. The day when spermatozoa were detected was designated day 1 of gestation. Each mated rat was individually housed in stainless steel rat racks. The study was divided into 24 replicates (randomized blocks) with each replicate containing one rat from each group. Cages within the replicates were assigned to one of four groups using computer-generated random number permutations. The individual animal numbers were then assigned sequentially within the relevant groups to give the rack plan (Appendix F). On arrival (gd 1), each rat was allocated to a cage (and therefore a treatment group) randomly within the replicate and individually identified by ear punching with the number assigned to it from the experimental design. Replicates were filled sequentially and 3 replicates were added to the study on each of the eight days on which rats were received.

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Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	24(1-24)
Low Dose	5	24(25-48)
Mid Dose	15	24(49-72)
High Dose	40	24(73-96)

Dosing:

The test substance was mixed with deionized water to provide dosing formulations with concentrations of 0.5, 1.5 and 4.0 mg/ml (adjusted for purity). All doses were administered by gavage in a volume of 1 ml/100g of body weight/day prepared prior to dosing the animals and stored at room temperature. Doses were adjusted daily to account for changes in bodyweight. Dosing was performed in group order with all animals receiving the same dose level being dosed sequentially. Dosing was performed in the morning of each day and the dosing formulations were stirred. The dosing suspensions were analyzed for concentration, homogeneity and stability (see attached Tables 2-4).

For the dosing formulations prepared for the study, the mean percent of nominal concentration was acceptable and varied from 96% to 102% (Table 2, attached). Homogeneity of quadruplicate samples taken from the bottom, middle and top of a 0.1 mg/ml nominal concentration formulation, which was not used to dose the animals of this study, was acceptable, ranging from -1.2% of deviation to +2.4% deviation from the overall mean concentration (Table 3, attached). Stability was satisfactory over a 26 day period (a period in excess of the period of compound administration) with duplicate samples at day 26 giving 110.7 percent of the initial analyzed concentrations (Table 4, attached). Additional data from analyses of concentrations of 1 and 10 mg/ml from a previously conducted study also indicated acceptable chemical stability and homogeneity (see report, Appendix D).

Observations

Clinical observations were performed daily. Body weights were recorded on gd 4, 7-16, and 22, and 3-day food consumption was measured on gds 4, 7, 10, 13, 16, 19, and 22. On day 22 of gestation the animals were killed and examined. The uterus (gravid and apparently nongravid) was removed, weighed and examined (along with ovaries) for implantations, fetuses and corpora lutea. Uteri without clear evidence of implantation were stained with ammonium sulfide. Live fetuses, early and late resorptions were evaluated for each uterine horn. Each fetus was weighed and individually identified. Percentage pre- and post-implantation loss were determined. Fetuses were examined for external and internal abnormalities, sexed, eviscerated and fixed in methanol. After 24 hours fixation, the skull was cut along the fronto-parietal suture line and the brain examined. The carcasses were then processed and stained in Alizarin

Red S for skeletal examinations. Individual bones on the front and hind limbs (manus and pes) were assessed and converted to a six point scale (see Appendix G of report, page 69).

Historical control data were not provided.

Statistical analysis

Data for animals that were non-pregnant or died were excluded from the statistical analysis. Maternal bodyweight was evaluated by ANOCOVA on initial (day 7) bodyweight. Food consumption, numbers of implantations, live fetuses/dam, gravid uterine weight, litter weight, mean fetal weights/litter and mean manus and pes scores/litter were evaluated by ANOVA. Maternal performance data, proportion of fetuses with each manus and pes score, and the proportion of fetuses or litters with each defect were assessed by the Fisher's Exact Test. Pre-implantation loss, post-implantation loss, early and late resorptions, male fetuses, and levelopmental anomalies were analyzed by: 1) percentages were analyzed by ANOVA following double arcsine transformation of Freeman and Tukey (1950) and 2) the proportion of fetuses or litters affected were evaluated by Fisher's Exact Test. All statistical tests were two-sided.

Compliance

A signed Statement of No Confidentiality Claim was provided.

A signed Flagging Statement indicating no adverse effects (40 CFR 158.34) was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

C. Results

Rationale for Selection of Dose Levels

No dose range-finding study was included in the report.

1. Maternal Toxicity

Mortality (see attachment, Table 5)

Two dams at the HDT died during dosing, one each on gd day 12 (killed in extremis) and gd 13 (found dead).

Clinical Observations

The major clinical sign observed in four of the 40 mg/kg/day dams related to the respiratory system and included abnormal noise, labored breathing, gasping; in the one dam which died in extremis there were signs of reduced breathing rate, irregular breathing, croaking and gasping.

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Body Weight (see attached Table 7, 10)

During dosing (days 8-16 inclusive) there was a modest (4-6.5%), but consistent, statistically significant decrement in HDT mean body weights in comparison to the control values (Table 7 of report). This depression in mean body weights remained after cessation of exposure.

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period (1-7)	Dosing Period (7-16)	Post Dosing Period (16-22)	Entire Gestation Period (1-22)	Corrected Body Weight Gains Dosing P.[1] (7-16)	Entire[2] (1-22)
Control	32.0	45.4	72.4	149.8	-34.3	70.1
LDT	34.9	46.5	74.3	155.7	-36.7	72.5
MDT	31.3	44.7	74.3	150.3	-38.9	66.7
HDT	31.8	28.1	75.8	135.7	-50.1	57.5

[1] = corrected mean body weight gain for dosing period = mean body weight gain for dosing period minus mean gravid uterus weight; may not be a fully valid correction since uterus has not completely developed on gd 16.
[2] = corrected mean body weight gain for entire gestation period = mean body weight gain for entire gestation period minus mean gravid uterus weight.
a = Data calculated by reviewer from attached Tables 7, 10 (ZENECA study no. CTL/P/3921)

Mean body weight gains reflected the depressed body weights with the body weight gains during dosing in the HDT attaining only 62% of control values (28.1 gms/HDT vs 45.4 gm/controls; Table I). Mean body weights corrected for gravid uterine weights were consistent with a compound-induced maternal toxicity at the HDT.

Food Consumption

The investigators supplied the following data:

Table II: Selected Food Consumption Data (g/animal/day)[a]

Group:	Prior to Dosing Period (4-7)	Dosing Period (7-10)	Dosing Period (13-16)	Post-Dosing Period (16-19)
Control	26.0	26.8	31.5	33.6
LDT	26.4	27.0	32.1	33.9
MDT	26.7	26.7	31.2	33.6
HDT	26.0	22.4**	27.8**	31.8

[a] = Data extracted from (Table 8; ZENECA study no. CTL/P/3921)
* Significantly different from controls; $p \leq 0.01$

DAS 1/16

Statistically significant decreases in food consumption were reported at the HDT when compared to control consumption during gds 7-10, 10-13 and 13-16 (see Table II above and attached Table 8). Food consumption following cessation of exposure at the HDT was slightly increased but did not exhibit a strong rebound effect. This may relate to the negative effect of the compound on the well-being of the dams at the HDT since several animals exhibited overt clinical signs of systemic toxicity.

Gross Pathological Observations

Of the two HDT dams which died during dosing, one had red areas in the lungs and distended stomach (2 dams) or sloughing of the stomach mucosa when washed (1 dam) was also noted (Table 9 of the report). The stomach findings appear to be related to an irritant effect of MTI. One of the HDT dams surviving to termination had a raised area in the stomach.

Other than the stomach, other gross necropsy findings of a dose- or compound-related nature were noted in animals completing the study. Incidental findings included pelvic dilatation of the kidneys, distended rectum and discolored uterine contents.

Cesarean section Observations (see attached Table 7)

A summary of selected C-section observations is presented below in Table III.

Aside from maternal wastage (deaths) at the HDT, there were no apparent dose-related effects upon mean implantations/dam, live fetuses/dam, resorptions/dam (early, late), preimplantation loss, the sex ratios or mean fetal weights.

Table III: Cesarean Section observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Mated	24	24	24	24
#Animals Pregnant	24	23	24	24
Pregnancy Rate (%)	100	96	100	100
Maternal Wastage				
#Died	0	0	0	2
#Died/pregnant	0	0	0	2
#Non pregnant	0	1	0	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	337	328	355	311
Corpora Lutea/dam	14.0	14.3	14.8	14.1
Total Implantation	285	283	299	258
Implantations/Dam	11.9	12.3	12.5	11.7
Total Live Fetuses	273	274	290	247
Live Fetuses/Dam	11.4	11.9	12.1	11.2
Total Resorptions	12	9	9	11
Early	11	8	6	9
Late	1	1	3	2
Resorptions/Dam	0.5	0.39	0.38	0.46
Mean Fetal Weight (gm)	4.88	4.89	4.87	4.92
Preimplantation Loss(%)	15.5	13.7	16.0	17.1
Postimplantation Loss(%)	3.5	3.4	3.6	4.7
Sex Ratio (% Male)	47.6	53.3	53.6	52.7

[a] = Data extracted from Tables 5,10; ZENECA study no. CTL/P/3921)

2. Developmental Toxicity

Table IV: Selected External/Visceral Anomalies

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Minor external/visceral defects				
#fetuses(litters)examined	273(24)	274(23)	290(24)	247(22)
#fetuses(litters)affected*	2(1)	2(2)	2(2)	3(3)
dilated ureter (slightly)	2(1)[a]	2(2)	2(2)	1(1)
External/visceral variants				
#fetuses(litters)examined	273(24)	274(23)	290(24)	247(22)
#fetuses(litters)affected*	18(9)	15(11)	14(10)	19(9)
kinked ureter	18(9)	15(11)	14(10)	19(9)

(+) Table 11 and 13; ZENECA study no. CTL/P/3921
(a) fetal [litter] incidence
* no statistical significance observed

No treatment or dose-related effects upon external or visceral defects were noted (Table IV).

Selected developmental skeletal effects (termed minor skeletal defects or variations) were decreased in a statistically significant manner at the HDT and/or MDT: non-ossification of the ventral tubercle of the cervical vertebrae, partially ossified 5th and 6th lumbar transverse processes and non-ossification of the 5th sternebrae (Table V). These are not considered adverse effects and are not biologically significant. An increase in fully ossified 7th transverse process of the cervical vertebrae (statistically significant at the HDT) is an isolated phenomenon whose biological significance is uncertain. This is considered to be an incidental finding. There were no apparent compound- or dose-related effects of MTI on the mean manus (forelimb) and pes (hindlimb) scores per litter for rates of ossification (see attached Table 14).

Table V: Selected Developmental Skeletal Anomalies

Observations[+]	Control	Low Dose	Mid Dose	High Dose
Minor skeletal defects				
#fetuses(litters)examined	273(24)	274(23)	290(24)	247(22)
#fetuses(litters)affected	107(24)	87(22)	87*(22)	70*(19)
Cervical vertebrae				
ventral tubercle, not ossified	41(12)[a]	28(11)	19**(9)	17**(8)
transverse process fully ossified, 7th	0(0)	0(0)	4(4)	5*(3)
Transverse processes				
5th lumbar partially ossf.	6(2)	4(2)	0*(0)	0*(0)
6th lumbar partially ossf.	8(2)	5(2)	0**(0)	0*(0)
Sternebrae				
bipartite, 5th	46(21)	44(18)	43(16)	34(16)
5th, not ossified	13(8)	9(4)	4*(4)	3*(2)
Skeletal variants				
#fetuses(litters)examined	273(24)	274(23)	290(24)	247(22)
#fetuses(litters)affected	204(24)	206(23)	220(24)	182(22)
Odontoid				
not ossified	42(12)	58(14)	49(15)	40(15)
Sternebrae				
5th partially ossified	82(21)	89(20)	96(20)	61(19)
Extra Ribs				
14th-short length	7(4)	9(3)	16(10)	8(4)

(+) Table 11 and 13; ZENECA study no. CTL/P/3921
(a) fetal [litter] incidence
statistical significance observed:* ($p<0.05$), ** ($p<0.01$)

D. Discussion/Conclusions

a. Maternal Toxicity:

Maternal toxicity was established for the HDT by 1) the observation of 2 deaths observed on gds 12 and 13 (1 in extremis, 1 found dead), 2) clinical signs of toxicity in 4 dams (including the two which died) related to the respiratory system (abnormal noise, labored breathing, gasping, reduced breathing rate), 3) statistically significant depressions in mean body weights (gds 8-16 inclusive) of 4-6.5% which remained decreased following cessation of dosing, 4) a 62% decrement in mean body weight gain during the dosing period, and 5) statistically significant depressions in mean food consumption on gds 7-10, 10-13 and 13-16.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There were no compound-related effects upon deaths or resorptions.

ii. Altered Growth:

Fetal growth was not affected by MTI administration.

iii. Developmental Anomalies (malformations, variations):

There was no evidence of a compound-related effect upon any form of fetal development.

D. Study Deficiencies:

No dose range-finding study was submitted but this did not affect the scientific quality of the study. Cesarian sections were performed on gd 22 in this study (with the day of insemination defined as gd 1). The guideline 83-3 recommended termination day is gd 21; therefore the fetuses in this study were allowed to mature a full additional day of gestation. This was not considered to have compromised the study findings.

E. Core Classification: Core Minimum

EPA Reg. No. 10182-385

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MTI: DEVELOPMENTAL TOXICITY STUDY IN THE RAT

TABLE 3

DOSING FORMULATION HOMOGENEITY

Preparation Date: 29/ 7/92

Nominal Concn. (mg/ml)	Sampling Point	Analysed Concn. (mg/ml)				Mean Concn. (mg/ml)	Overall Mean Concn. (mg/ml)	% Deviation
0.1	BOTTOM	0.078	0.081	0.084	0.081	0.081		-1.2
	MIDDLE	0.088	0.073	0.084	0.081	0.082		+0.0
	TOP	0.089	0.083	0.082	0.081	0.084	0.082	+2.4

% Deviation = Deviation of mean concentration from overall mean concentration.

MTI: DEVELOPMENTAL TOXICITY STUDY IN THE RAT

TABLE 4

DOSING FORMULATION CHEMICAL STABILITY

Nominal Concn. (mg/ml)	Preparation Date	Analysis Date	Analysis Interval (Days)	Analysed Concn. (mg/ml)		Mean Concn. (mg/ml)	% of Initial Concn.
0.1	29/ 7/92	29/ 7/92	0	0.083	0.084	0.084	100.0
		24/ 8/92	26	0.093	0.092	0.093	110.7

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MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 5
INTERGROUP COMPARISON OF MATERNAL PERFORMANCE

	Dose Level of MTI (mg/kg/day)			
	0(Control)	5	15	40
Mated	24/24	24/24	24/24	24/24
Not pregnant	0/24	1/24	0/24	0/24
Pregnant	24/24	23/24	24/24	24/24
Intercurrent deaths	0/24	0/24	0/24	2/24
- live foetuses in utero	0/24	0/24	0/24	2/24
Live foetuses in utero at termination	24/24	23/24	24/24	22/24

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 7
INTERGROUP COMPARISON OF MATERNAL BODYWEIGHTS (g)

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
PRE-DOSING					
Day 1	MEAN	258.1	255.2	258.8	253.7
	S.D.	16.7	14.7	17.9	14.5
	N	24	23	24	22
Day 4	MEAN	276.5	276.1	278.6	271.8
	S.D.	16.0	15.9	18.5	14.3
	N	24	23	24	22
Day 7	MEAN	290.1	290.1	290.1	285.5
	S.D.	18.6	17.0	18.8	16.7
	N	24	23	24	22
DURING DOSING					
Day 8	MEAN	293.1	292.9	293.1	281.4**
	S.D.	18.9	16.5	18.4	17.1
	N	24	23	24	22
Day 9	MEAN	297.7	297.7	296.8	282.9**
	S.D.	19.6	17.6	18.6	16.8
	N	24	23	24	22
Day 10	MEAN	302.2	302.3	300.9	286.5**
	S.D.	19.9	16.8	17.7	17.8
	N	24	23	24	22
Day 11	MEAN	307.3	307.9	306.7	291.1**
	S.D.	20.7	16.8	18.2	17.2
	N	24	23	24	22

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MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 7 continued
INTERGROUP COMPARISON OF MATERNAL BODYWEIGHTS (g)

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
Day 12	MEAN	312.9	312.5	312.0	294.5**
	S.D.	21.2	17.6	18.8	17.1
	N	24	23	24	22
Day 13	MEAN	318.3	318.4	317.2	297.7**
	S.D.	21.3	17.5	20.7	18.5
	N	24	23	24	22
Day 14	MEAN	322.6	322.4	322.3	302.9**
	S.D.	21.8	17.6	19.7	17.7
	N	24	23	24	22
Day 15	MEAN	329.5	330.6	328.0	308.6**
	S.D.	22.6	18.2	19.6	17.9
	N	24	23	24	22
Day 16	MEAN	335.5	336.6	334.8	313.6**
	S.D.	23.2	18.6	20.3	17.7
	N	24	23	24	22
POST DOSING					
Day 19	MEAN	366.7	369.7	369.3	350.3**
	S.D.	29.9	22.1	24.4	21.4
	N	24	23	24	22
Day 22	MEAN	407.9	410.9	409.1	389.4*
	S.D.	33.6	26.8	27.8	23.5
	N	24	23	24	22

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 8
INTERGROUP COMPARISON OF MATERNAL FOOD CONSUMPTION (g/day)

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
PRE-DOSING					
Days 1-4	MEAN	23.9	24.0	24.4	23.0
	S.D.	1.6	1.7	2.1	2.1
	N	24	23	24	22
Days 4-7	MEAN	26.0	26.4	26.7	26.0
	S.D.	2.1	1.9	2.1	2.3
	N	24	23	24	22
DURING DOSING					
Days 7-10	MEAN	26.8	27.0	26.7	22.4**
	S.D.	2.2	1.8	2.3	3.2
	N	21	21	21	19
Days 10-13	MEAN	28.8	29.7	29.2	24.7**
	S.D.	2.6	2.0	2.7	4.2
	N	21	21	21	19
Days 13-16	MEAN	31.5	32.1	31.2	27.8**
	S.D.	2.7	2.5	3.2	3.8
	N	24	23	24	22
POST DOSING					
Days 16-19	MEAN	33.6	33.9	33.6	31.8
	S.D.	2.9	2.5	3.2	2.7
	N	24	23	24	22
Days 19-22	MEAN	33.4	33.7	32.8	32.1
	S.D.	3.3	3.5	4.7	3.5
	N	24	23	24	22

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MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 10
INTERGROUP COMPARISON OF LITTER DATA

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
Mean no. of corpora lutea					
	MEAN	14.0	14.3	14.8	14.1
	S.D.	1.9	1.0	1.7	1.8
	N	24	23	24	22
Mean no. of implantations					
	MEAN	11.9	12.3	12.5	11.7
	S.D.	3.3	3.1	3.1	3.5
	N	24	23	24	22
Mean no. of live foetuses					
	MEAN	11.4	11.9	12.1	11.2
	S.D.	3.0	3.1	3.3	3.7
	N	24	23	24	22
Mean gravid uterus weight (g)					
	MEAN	79.7	83.2	83.6	78.2
	S.D.	22.1	21.7	19.9	23.3
	N	24	23	24	22
Mean litter weight (g)					
	MEAN	55.7	58.4	58.8	54.9
	S.D.	17.2	17.0	16.1	17.8
	N	24	23	24	22
Mean foetal weight (g)					
	MEAN	4.88	4.89	4.87	4.92
	S.D.	0.57	0.52	0.45	0.52
	N	24	23	24	22
SEX DISTRIBUTION					
Prop. of male foetuses		132/273	146/274	156/290	136/247
Percentage	MEAN	47.6	53.3	53.6	52.7
	S.D.	18.6	9.5	16.2	19.4
	N	24	23	24	22

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MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 10 continued
INTERGROUP COMPARISON OF LITTER DATA

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
PRE-IMPLANTATION LOSS					
Prop. of implants affected		52/337	45/328	56/355	53/311
Percentage	MEAN	15.5	13.7	16.0	17.1
	S.D.	21.2	20.7	17.4	21.7
	N	24	23	24	22
Prop. of litters affected		16/24	12/23	18/24	13/22
POST-IMPLANTATION LOSS					
Prop. of implants affected		12/285	9/283	9/299	11/258
Percentage	MEAN	3.5	3.4	3.6	4.7
	S.D.	5.5	5.4	5.4	7.5
	N	24	23	24	22
Prop. of litters affected		8/24	8/23	9/24	8/22
EARLY INTRA-UTERINE DEATHS					
Prop. of implants affected		11/285	8/283	6/299	9/258
Percentage	MEAN	3.3	3.0	1.9	4.0
	S.D.	5.1	4.7	3.3	7.5
	N	24	23	24	22
Prop. of litters affected		8/24	8/23	6/24	6/22
LATE INTRA-UTERINE DEATHS					
Prop. of implants affected		1/285	1/283	3/299	2/258
Percentage	MEAN	0.3	0.4	1.8	0.7
	S.D.	1.4	1.7	5.0	2.4
	N	24	23	24	22
Prop. of litters affected		1/24	1/23	3/24	2/22

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 11
INTERGROUP COMPARISON OF FOETAL DEFECTS AND VARIANTS

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
MAJOR EXTERNAL/VISCERAL DEFECTS					
Prop. of foetuses affected		2/273	2/274	3/290	0/247
Percentage	MEAN	0.7	0.7	1.0	0.0
	S.D.	2.3	2.2	2.6	0.0
	N	24	23	24	22
Prop. of litters affected		2/24	2/23	3/24	0/22
MINOR EXTERNAL/VISCERAL DEFECTS ONLY					
Prop. of foetuses affected		2/273	2/274	2/290	3/247
Percentage	MEAN	0.6	0.7	0.8	1.1
	S.D.	3.1	2.2	2.7	2.9
	N	24	23	24	22
Prop. of litters affected		1/24	2/23	2/24	3/22
EXTERNAL/VISCERAL VARIANTS					
Prop. of foetuses affected		18/273	15/274	14/290	19/247
Percentage	MEAN	7.7	5.8	5.6	7.4
	S.D.	14.6	7.5	9.9	9.9
	N	24	23	24	22
Prop. of litters affected		9/24	11/23	10/24	9/22

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 11 continued
INTERGROUP COMPARISON OF FOETAL DEFECTS AND VARIANTS

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
MAJOR SKELETAL DEFECTS					
Prop. of foetuses affected		0/273	0/274	2/290	0/247
Percentage	MEAN	0.0	0.0	0.6	0.0
	S.D.	0.0	0.0	2.1	0.0
	N	24	23	24	22
Prop. of litters affected		0/24	0/23	2/24	0/22
MINOR SKELETAL DEFECTS ONLY					
Prop. of foetuses affected		107/273	87/274	87/290*	70/247*
Percentage	MEAN	39.1	32.1	28.1	28.9*
	S.D.	23.5	25.6	21.8	22.1
	N	24	23	24	22
Prop. of litters affected		24/24	22/23	22/24	19/22
SKELETAL VARIANTS					
Prop. of foetuses affected		204/273	206/274	220/290	182/247
Percentage	MEAN	74.1	76.7	75.2	72.5
	S.D.	25.4	26.3	24.9	28.8
	N	24	23	24	22
Prop. of litters affected		24/24	23/23	24/24	22/22

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
EXTERNAL/VISCERAL DEFECTS									
TORSO									
SITUS INVERSUS TOTALIS	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
EYES									
ANOPHTHALMIA	MAJ	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
MICROPHTHALMIA	MAJ	1	(0.4%)	2	(0.7%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	2	(8.7%)	0	(0.0%)	0	(0.0%)
BRAIN									
INTERNAL HYDROCEPHALY	MAJ	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
ABDOMEN									
ANAL ATRESIA	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
LIVER									
CYST(S) ATTACHED	MIN	0	(0.0%)	0	(0.0%)	0	(0.0%)	2	(0.8%)
		0	(0.0%)	0	(0.0%)	0	(0.0%)	2	(9.1%)
KIDNEY									
DISPLACED	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
KIDNEY									
ABSENT	MAJ	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
URETER									
DISPLACED	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
DILATED - SLIGHTLY	MIN	2	(0.7%)	2	(0.7%)	2	(0.7%)	1	(0.4%)
		1	(4.2%)	2	(8.7%)	2	(8.3%)	1	(4.5%)
KINKED	VAR	18	(6.6%)	15	(5.5%)	14	(4.8%)	19	(7.7%)
		9	(37.5%)	11	(47.8%)	10	(41.7%)	9	(40.9%)
ABSENT	MAJ	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
TAIL									
ABSENT	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
SKELETAL DEFECTS									
SKULL									
INTERPARIETAL - PARTIALLY OSSIFIED	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
NASALS INCLINED TO MID SUTURE LINE	MIN	2	(0.7%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
OCCIPITAL - PARTIALLY OSSIFIED	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
PARIETALS - PARTIALLY OSSIFIED	MIN	3	(1.1%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
SKULL:SUTURAL BONES									
BETWEEN INTERPARIETAL AND PARIETALS	MIN	1	(0.4%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
BETWEEN PARIETALS	MIN	0	(0.0%)	0	(0.0%)	2	(0.7%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	2	(8.3%)	0	(0.0%)
SKULL:FONTANELLE									
ANTERIOR - WIDENED SLIGHTLY	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
ODONTOID									
NOT OSSIFIED	VAR	42	(15.4%)	58	(21.2%)	49	(16.9%)	40	(16.2%)
		12	(50.0%)	14	(60.9%)	15	(62.5%)	15	(68.2%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
CERVICAL VERTEBRAE									
ARCH ABSENT,6TH	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
ARCH PARTIALLY OSSIFIED,3RD	MIN	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(0.4%)
		0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(4.5%)
ARCH PARTIALLY OSSIFIED,4TH	MIN	1	(0.4%)	2	(0.7%)	0	(0.0%)	2	(0.8%)
		1	(4.2%)	2	(8.7%)	0	(0.0%)	1	(4.5%)
ARCH PARTIALLY OSSIFIED,5TH	MIN	5	(1.8%)	1	(0.4%)	2	(0.7%)	3	(1.2%)
		3	(12.5%)	1	(4.3%)	2	(8.3%)	1	(4.5%)
ARCH REDUCED,4TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
NOT OSSIFIED, VENTRAL TUBERCLE	MIN	41	(15.0%)	28	(10.2%)	19**	(6.6%)	17**	(6.9%)
		12	(50.0%)	11	(47.8%)	9	(37.5%)	8	(36.4%)
CENTRUM NOT OSSIFIED,2ND	VAR	79	(28.9%)	73	(26.6%)	87	(30.0%)	83	(33.6%)
		18	(75.0%)	17	(73.9%)	21	(87.5%)	19	(86.4%)
CENTRUM NOT OSSIFIED,4TH	MIN	19	(7.0%)	11	(4.0%)	13	(4.5%)	10	(4.0%)
		7	(29.2%)	3	(13.0%)	4	(16.7%)	5	(22.7%)
CENTRUM NOT OSSIFIED,5TH	MIN	8	(2.9%)	3	(1.1%)	6	(2.1%)	8	(3.2%)
		5	(20.8%)	2	(8.7%)	3	(12.5%)	2	(9.1%)
CENTRUM NOT OSSIFIED,6TH	MIN	3	(1.1%)	2	(0.7%)	3	(1.0%)	0	(0.0%)
		2	(8.3%)	2	(8.7%)	2	(8.3%)	0	(0.0%)
CENTRUM NOT OSSIFIED,7TH	MIN	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
CENTRUM NOT OSSIFIED,3RD	VAR	33	(12.1%)	20	(7.3%)	27	(9.3%)	28	(11.3%)
		10	(41.7%)	5	(21.7%)	9	(37.5%)	10	(45.5%)
TRANSVERSE PROCESS FULLY OSSIFIED,7TH	MIN	0	(0.0%)	0	(0.0%)	4	(1.4%)	5*	(2.0%)
		0	(0.0%)	0	(0.0%)	4	(16.7%)	3	(13.6%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
CERVICAL VERTEBRAE									
TRANSVERSE PROCESS PARTIALLY OSSIFIED,7TH	VAR	35	(12.8%)	33	(12.0%)	34	(11.7%)	23	(9.3%)
		16	(66.7%)	18	(78.3%)	17	(70.8%)	11	(50.0%)
THORACIC VERTEBRAE									
CENTRUM BIPARTITE,2ND	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
CENTRUM BIPARTITE,4TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
CENTRUM BIPARTITE,5TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
CENTRUM BIPARTITE,9TH	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
CENTRUM BIPARTITE,10TH	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
CENTRUM BIPARTITE,11TH	MIN	2	(0.7%)	0	(0.0%)	0	(0.0%)	2	(0.8%)
		2	(8.3%)	0	(0.0%)	0	(0.0%)	2	(9.1%)
CENTRUM BIPARTITE,12TH	MIN	3	(1.1%)	1	(0.4%)	3	(1.0%)	2	(0.8%)
		1	(4.2%)	1	(4.3%)	3	(12.5%)	2	(9.1%)
CENTRUM BIPARTITE,13TH	MIN	1	(0.4%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
CENTRUM PARTIALLY OSSIFIED,4TH	MIN	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
CENTRUM PARTIALLY OSSIFIED,11TH	MIN	0	(0.0%)	0	(0.0%)	2	(0.7%)	2	(0.8%)
		0	(0.0%)	0	(0.0%)	2	(8.3%)	2	(9.1%)
CENTRUM PARTIALLY OSSIFIED,12TH	MIN	1	(0.4%)	0	(0.0%)	1	(0.3%)	2	(0.8%)
		1	(4.2%)	0	(0.0%)	1	(4.2%)	2	(9.1%)

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MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
THORACIC VERTEBRAE									
CENTRUM PARTIALLY OSSIFIED,13TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	1	(0.4%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	1	(4.5%)
HEMICENTRUM NOT OSSIFIED,13TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
HEMICENTRUM PARTIALLY OSSIFIED,2ND	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
HEMICENTRUM PARTIALLY OSSIFIED,5TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
HEMICENTRUM PARTIALLY OSSIFIED,12TH	MIN	1	(0.4%)	0	(0.0%)	1	(0.3%)	1	(0.4%)
		1	(4.2%)	0	(0.0%)	1	(4.2%)	1	(4.5%)
LUMBAR VERTEBRAE									
CENTRUM BIPARTITE,1ST	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
CENTRUM PARTIALLY OSSIFIED,1ST	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
HEMICENTRUM PARTIALLY OSSIFIED,1ST	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
TRANSVERSE PROCESSES									
OF 4TH LUMBAR FULLY OSSIFIED	VAR	12	(4.4%)	18	(6.6%)	13	(4.5%)	20	(8.1%)
		9	(37.5%)	8	(34.8%)	10	(41.7%)	7	(31.8%)
OF 5TH LUMBAR PARTIALLY OSSIFIED	MIN	6	(2.2%)	4	(1.5%)	0*	(0.0%)	0*	(0.0%)
		2	(8.3%)	2	(8.7%)	0	(0.0%)	0	(0.0%)
OF 6TH LUMBAR PARTIALLY OSSIFIED	MIN	8	(2.9%)	5	(1.8%)	0**	(0.0%)	0*	(0.0%)
		2	(8.3%)	2	(8.7%)	0	(0.0%)	0	(0.0%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
CAUDAL VERTEBRAE									
ABSENT	MAJ	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
VERTEBRAL COLUMN									
27 PRE-SACRAL VERTEBRAE	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
STERNEBRAE									
BIPARTITE,1ST	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
BIPARTITE,2ND	MIN	1	(0.4%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
BIPARTITE,3RD	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
BIPARTITE,4TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
BIPARTITE,5TH	MIN	46	(16.8%)	44	(16.1%)	43	(14.8%)	34	(13.8%)
		21	(87.5%)	18	(78.3%)	16	(66.7%)	16	(72.7%)
BIPARTITE,6TH	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
MISALIGNED EXTREMELY,2ND	MIN	1	(0.4%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		1	(4.2%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
MISALIGNED EXTREMELY,3RD	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
MISALIGNED EXTREMELY,4TH	MIN	0	(0.0%)	0	(0.0%)	2	(0.7%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	2	(8.3%)	0	(0.0%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
STERNEBRAE									
MISALIGNED EXTREMELY,5TH	MIN	1	(0.4%)	3	(1.1%)	2	(0.7%)	0	(0.0%)
		1	(4.2%)	3	(13.0%)	2	(8.3%)	0	(0.0%)
NOT OSSIFIED,5TH	MIN	13	(4.8%)	9	(3.3%)	4*	(1.4%)	3*	(1.2%)
		8	(33.3%)	4	(17.4%)	4	(16.7%)	2	(9.1%)
PARTIALLY OSSIFIED,1ST	MIN	1	(0.4%)	0	(0.0%)	0	(0.0%)	1	(0.4%)
		1	(4.2%)	0	(0.0%)	0	(0.0%)	1	(4.5%)
PARTIALLY OSSIFIED,2ND	MIN	0	(0.0%)	0	(0.0%)	3	(1.0%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	3	(12.5%)	0	(0.0%)
PARTIALLY OSSIFIED,4TH	MIN	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
PARTIALLY OSSIFIED,5TH	VAR	82	(30.0%)	89	(32.5%)	96	(33.1%)	61	(24.7%)
		21	(87.5%)	20	(87.0%)	20	(83.3%)	19	(86.4%)
PARTIALLY OSSIFIED,6TH	MIN	3	(1.1%)	9	(3.3%)	0	(0.0%)	0	(0.0%)
		2	(8.3%)	3	(13.0%)	0	(0.0%)	0	(0.0%)
MISALIGNED SLIGHTLY,2ND	MIN	2	(0.7%)	3	(1.1%)	2	(0.7%)	0	(0.0%)
		2	(8.3%)	3	(13.0%)	2	(8.3%)	0	(0.0%)
MISALIGNED SLIGHTLY,3RD	MIN	3	(1.1%)	3	(1.1%)	3	(1.0%)	1	(0.4%)
		3	(12.5%)	3	(13.0%)	3	(12.5%)	1	(4.5%)
MISALIGNED SLIGHTLY,4TH	MIN	7	(2.6%)	3	(1.1%)	7	(2.4%)	2	(0.8%)
		6	(25.0%)	3	(13.0%)	6	(25.0%)	2	(9.1%)
MISALIGNED SLIGHTLY,5TH	MIN	6	(2.2%)	10	(3.6%)	12	(4.1%)	5	(2.0%)
		4	(16.7%)	8	(34.8%)	10	(41.7%)	3	(13.6%)
FUSED STERNEBRAE									
1ST AND 2ND	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 13 continued
INTERGROUP COMPARISON OF FOETAL DEFECT INCIDENCE

		Dose Level of MTI (mg/kg/day) 0(Control)		5		15		40	
FUSED STERNEBRAE									
3RD AND 4TH	MIN	0	(0.0%)	1	(0.4%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	1	(4.2%)	0	(0.0%)
4TH AND 5TH	MIN	0	(0.0%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		0	(0.0%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
RIBS									
FLOATING,1ST	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
FLOATING,2ND	MIN	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
KINKED,10TH	MIN	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(0.4%)
		0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(4.5%)
KINKED,11TH	MIN	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(0.4%)
		0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(4.5%)
SHORT,13TH	MIN	1	(0.4%)	1	(0.4%)	0	(0.0%)	0	(0.0%)
		1	(4.2%)	1	(4.3%)	0	(0.0%)	0	(0.0%)
EXTRA RIBS									
14TH - NORMAL LENGTH	VAR	0	(0.0%)	0	(0.0%)	1	(0.3%)	0	(0.0%)
		0	(0.0%)	0	(0.0%)	1	(4.2%)	0	(0.0%)
14TH - SHORT LENGTH	VAR	7	(2.6%)	9	(3.3%)	16	(5.5%)	8	(3.2%)
		4	(16.7%)	3	(13.0%)	10	(41.7%)	4	(18.2%)
CALCANEUM									
NOT OSSIFIED	VAR	135	(49.5%)	133	(48.5%)	148	(51.0%)	138	(55.9%)
		21	(87.5%)	21	(91.3%)	21	(87.5%)	21	(95.5%)

MTI : DEVELOPMENTAL TOXICITY STUDY IN THE RAT
TABLE 14
INTERGROUP COMPARISON OF MANUS/PES ASSESSMENT

		Dose Level of MTI (mg/kg/day) 0(Control)	5	15	40
MANUS SCORES					
Prop. with score 2		2 (0.7%)	2 (0.7%)	0 (0.0%)	0 (0.0%)
Prop. with score 3		23 (8.4%)	21 (7.7%)	7 (2.4%)	9 (3.6%)
Prop. with score 4		181 (66.3%)	186 (67.9%)	230 (79.3%)	178 (72.1%)
Prop. with score 5		67 (24.5%)	65 (23.7%)	53 (18.3%)	60 (24.3%)
Mean manus score per litter	MEAN	4.17	4.17	4.17	4.21
	S.D.	0.41	0.48	0.31	0.38
	N	24	23	24	22
PES SCORES					
Prop. with score 3		11 (4.0%)	10 (3.6%)	4 (1.4%)	4 (1.6%)
Prop. with score 4		73 (26.7%)	60 (21.9%)	47 (16.2%)	31 (12.6%)
Prop. with score 5		178 (65.2%)	195 (71.2%)	239 (82.4%)	212 (85.8%)
Prop. with score 6		11 (4.0%)	9 (3.3%)	0 (0.0%)	0 (0.0%)
Mean pes score per litter	MEAN	4.71	4.76	4.80	4.81
	S.D.	0.47	0.41	0.29	0.32
	N	24	23	24	22

011613

MTD UDS

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED (7509C)

Signature: Nancy E. McCarroll
Date: 3-28-95
Signature: James N. Rowe
Date: 3/28/95

DATA EVALUATION REPORT

STUDY TYPE: In vivo/in vitro unscheduled DNA synthesis assay in primary rat hepatocytes following in vivo dosage.

TOX CHEM NUMBER:

PC CODE:

MRID NUMBER: 431387-31

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYM(S): MTI

STUDY NUMBER(S): CTL/P/3134

SPONSOR: Zeneca Inc., Wilmington, DE

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield. Cheshire, UK

TITLE OF REPORT: 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-one (MTI): Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo

AUTHOR(S): J. C. Kennelly

REPORT ISSUED: November 28, 1990

CONCLUSION(S) - Executive Summary:

In an in vivo/in vitro unscheduled DNA synthesis (UDS) study (MRID No. 431387-31), groups of five male rats were administered single oral gavage doses of 76, 117, or 180 mg/kg MTI prepared in deionized water. The high dose was estimated to be 80% of the male rat oral LD_{50}. Animals were sacrificed at 4 and 12 hours posttreatment and recovered hepatocytes were scored for UDS Two independent trials were performed.

Clinical signs of toxicity noted immediately after dosing included salivation, difficulty in breathing and staining and fluid around the nose. Treatment with MTI produced no evidence of cytotoxicity for the target cells. There was also no evidence of a genotoxic response at any dose or sacrifice time.

CLASSIFICATION: Acceptable.

This study is classified as acceptable. It satisfies the guideline requirement for a UDS assay (84-4).

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A. MATERIALS:

1. Test Material: MTI

Description: Light brown (buff) solid
Identification number: Batch Number MTIBP1 ex Grangemouth works
Purity: 95%
Receipt date: July 1989
Stability: Expiration date February 28, 1991
Contaminants: None listed
Vehicle used: Deionized water
Other provided information: The test material was stored in a sealed container at room temperature. Test material dose formulations were prepared as dispersions in the vehicle and were used within 24 hours of preparation. Analytical determinations to verify actual concentrations were not performed and solutions were not corrected to 100% a. i.

2. Control Substances:

Vehicle control/concentration/route of administration: Deionized water 10 ml/kg by oral gavage.

Positive controls/concentration/route of administration: N-Nitroso-dimethylamine (DMN) dissolved in deionized water was administered by oral gavage at a final dose of 10 mg/kg (4-hour sacrifice) and 6-p-dimethylaminophenylazobenzthiazole (6-BT) was prepared in corn oil and administered by oral gavage at a final concentration of 40 mg/kg (12-hour sacrifice).

3. Medium: WME: Williams' Medium E containing 4 mM L-glutamine and antibiotics; WME+: Williams' Medium E as above supplemented with 10% fetal bovine serum (FBS).

4. Test Compound:

Route of administration: Once by oral gavage (dosing volume = 10 ml/kg).

Dose levels:
Preliminary Toxicity Test: 117 and 180 mg/kg (5 males/group)

Note: The male rat oral LD_{50} was reported to be ≈224 mg/kg.

UDS Assay: 76, 117, and 180 mg/kg

Note: Two independent trials of the UDS assay were performed.

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5. Test Animals:

(a) Species: Rat; Strain: Alderley Park (Alpk:APfSD); Age (at dosing): 6-8 weeks; Sex: Males; Weight range (at dosing): 188-236-262 g; Source: ICI Barriered Animal Breeding Unit, ICI Pharmaceuticals.

(b) Number of animals/dose:

Preliminary toxicity test: 5 males/group

UDS assay:

- Treatment groups: 10 males (5/sacrifice time)
- Positive controls: 2 males (1 animal/positive control/sacrifice time)
- Vehicle control: 4 males (2/sacrifice time)

(c) Properly maintained? Yes.

B. TEST PERFORMANCE

1. UDS Assay:

(a) Perfusion techniques/hepatocyte harvest: At ≈4 and 12 hours postdosing, animals in the appropriate test material, vehicle or positive control groups were anesthetized with Fluothane and livers were perfused with buffer solutions and a collagenase solution (75 U/ml). Livers were removed and finely chopped; the resulting crude homogenate was diluted with WME, filtered and centrifuged. Pellets were resuspended in WEM+ and cell densities were adjusted to 1.5×10^5 viable cells/ml. Prepared hepatocytes in 3-ml volumes were plated onto coverslips placed in 6-well culture dishes. Six coverslips were made per suspension. Cultures were allowed to attach at 37°C with 5% CO_2 for 1.5-2.5 hours. Unattached cells were removed. Viable cells were incubated in fresh WME containing ^{3}H-thymidine (1 μCi/ml) for 4 hours, washed and reincubated overnight in WME containing unlabeled thymidine.

(b) Slide preparation: Hepatocytes attached to coverslips were washed, fixed in glacial acetic acid:absolute alcohol (1:3), washed, dried and mounted.

(c) Preparation of autoradiographies/grain development: Slides were coated with Ilford K-2 emulsion, exposed at -4°C in the dark for 14 days, developed in Kodak D19, stained with Meyers Haemalum and eosin Y phloxine and coded.

(d) Grain counting: Hepatocytes harvested from animals that were sacrificed at 4 and 12 hours postexposure were used to determine UDS. When possible, the grains of at least 100 morphologically normal cells (50/slide/animal) were counted. To determine the net nuclear grains (N-C), grains were counted in a nuclear-sized area within the most heavily labeled cytoplasmic area adjacent to each nucleus. The cytoplasmic grain count (C), was then subtracted from

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the nuclear grain count (N) of that cell. The percentage of cells in repair (i.e., cells with at least five N-C) was also calculated. Mean gross nuclear counts, mean cytoplasmic grain counts as well as the mean and standard deviation of the mean for net nuclear counts were calculated.

(e) Statistical methods: The UDS data were not evaluated for statistical significance.

2. Evaluation Criteria:

(a) Study validity: The study was considered acceptable if (1) the mean N-C for the vehicle control group was <0 N-C and (2) N-C for the positive control groups was ≥5 N-C with at least 20% of the cells in repair.

(b) Positive response: The assay was considered positive if the mean N-C count for any treatment group was ≥5 net nuclear grains and the percentage of cells in repair was ≥20%.

C. REPORTED RESULTS:

1. Rationale for Dose Selection/Preliminary Toxicity Test: The study author stated that from the findings of an acute oral toxicity study (Report No. CTL/P/2791, ICI Central Toxicology Laboratory) the male rat oral LD_{50} was ≈224 mg/kg. Accordingly, doses used in the toxicity test (117 and 180 mg/kg) were estimated to be 50 and 80% of the LD_{50}, respectively. Groups of five male rats received single oral gavage administrations of the selected test material doses and were observed for clinical signs of compound toxicity for 4 days. Reported results indicated that no deaths occurred in the treated animals, and the investigators set 180 mg/kg as the maximum tolerated dose (MTD).

2. UDS Assay: Based on the findings of the preliminary toxicity test, groups of five male rats were administered single oral gavage doses of 76, 117, or 180 mg/kg MTI. Clinical signs of toxicity noted immediately following dosing included salivation, breathing difficulties and staining with fluid around the nose. With the exception of a single rat, clinical signs subsided or disappeared prior to sacrifice. Results from the analysis of hepatocytes prior to autoradiography indicated that treatment with MTI for either 4 or 12 hours was not cytotoxic. Slides prepared from animals exposed to 117 or 180 mg/kg of the test material were, therefore, selected for the assessment of UDS induction. No appreciable increases in N-C or the percentage of cells in repair were noted in the hepatocytes harvested from the male rats treated with MTI at either sacrifice time. Similar results were obtained in the independently performed repeat trial. By contrast to the negative findings with the test material, treatment of single animals with either of the positive controls (DMN or 6-BT) resulted in marked increases in N-C as well as the percentage of cells in repair in both trials. Summarized results of the data combined for both trials are shown in study Table 1 (see Appendix I).

The study author concluded, therefore, that MTI did not induce DNA repair in the hepatocytes of rats treated in vivo.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author correctly interpreted the data. MTI was

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tested to the MTD in two independently performed in vivo/in vitro rat hepatocyte UDS assays, and failed to induce either a cytotoxic or genotoxic response in the hepatocytes of male rats harvested 4 or 12 hours post-treatment. Results with the positive controls (DMN at 10 mg/kg and a 4-hour harvest or 6-BT at 40 mg/ml and a 12-hour harvest) demonstrated that the assay was sufficiently sensitive to detect genotoxicity. We conclude, therefore, that the study provided acceptable evidence that MTI was negative in this whole animal test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A signed and dated Quality Assurance Statement was provided.)

F. Appendix attached? YES; Appendix I--Study Summary Table

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_____ Identity of product inert ingredients.

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_____ Sales or other commercial/financial information.

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_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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MTI

MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED

Signature: Nancy L. McCarroll
Date: 3-28-95

EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED

Signature: James N. Rowe
Date: 3/28/95

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture cytogenetic assay in human lymphocytes

Tox Chem. Number:

PC Code:

MRID Number: 431387-30

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYM: MTI

STUDY NUMBER: CTL/P/3035

SPONSOR: Zeneca Inc., Wilmington, DE

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-one (MTI): An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes

AUTHORS: Jones, K. and Mackay, J. M.

REPORT ISSUED: July 12, 1990

CONCLUSIONS-EXECUTIVE SUMMARY: In an in vitro cytogenetic assay (MRID No. 431387-30), cultured human lymphocytes, obtained from one male and one female donor were exposed to MTI doses of 2, 10, or 20 μg/ml -S9 (male donor); 1, 5, or 10 μg/ml -S9 (female donor) or 2, 10, or 20 μg/ml +S9 (both donors). The test material was delivered to the test system in dimethyl sulfoxide, and the S9 was derived from Aroclor 1254 induced rat liver.

Significant ($p<0.01$) clastogenic effects were seen at 10 and 20 μg/ml without S9 activation and at 20 μg/ml with S9 activation in lymphocytes derived from the male donor and at 10 and 20 μg/ml +S9 in cells derived from the female donor. Higher levels with or without S9 activation were severely cytotoxic.

MTI is considered positive for the induction of structural chromosome aberrations in cultured human lymphocytes.

CLASSIFICATION: The study is classified as acceptable. It satisfies the guideline requirement for an in vitro cytogenetic study (§84-2).

A. MATERIALS:

1. Test Material: MTI

Description: Buff colored solid
Identification No.: Reference numbers: MTIBP1
Purity: 95.0%
Receipt date: Not reported
Stability: Unspecified
CAS number: None listed
Solvent used: Dimethyl sulphoxide (DMSO)
Other provided information: The test material was stored at room temperature in the dark. The frequency of dose solution preparation was not provided. Analytical determinations were not performed to verify actual concentrations used in the study; solutions were not adjusted to 100% a.i.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/5 µL/ml

Positive: Nonactivation (concentrations, solvent): Mitomycin C (Mit C) was prepared in deionized water to yield a final concentration of 1.0 µg/ml.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in deionized water to yield a final concentration of 50 µg/ml.

3. Activation: S9 derived from male AlpK:APfSD

__x__ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat S9 liver homogenate was prepared by the performing laboratory.

S9 mix composition:

Component	Final Concentration in S9 Mix
Na_2HPO_4	75 mM
KCl	25 mM
NADP	3 mM
Glucose 6-Phosphate	4 mM
$MgCl_2$	6 mM
S9	50%

Note: 200 µl of the S9 mix were added to 10 ml of culture medium.

4. Test Compound Concentration Used:

   (a) Preliminary cytotoxicity assay: Two cytotoxicity assays were conducted; doses used were: 1, 5, 20, 110, and 550 µg/ml +/-S9 (Trial 1) and 1, 2, 5, 10, and 20 µg/ml +/-S9 (Trial 2).

   (b) Cytogenetic assay: Cultures from Trial 2 of the cytotoxicity assay that were exposed to 2, 10, or 20 µg/ml -S9 (male donor) and 1, 5, or 10 µg/ml -S9 (female donor) or 2, 10, or 20 µg/ml +/-S9 (both donors) were scored for structural aberrations.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy non-smoking subjects (one male and one female) with established histories of a low incidence of chromosome damage. Lymphocyte cultures were initiated and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, an unspecified concentration of phytohemagglutinin, and antibiotics.

   Properly maintained? Yes.

   Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

   Cell line or strain periodically checked for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

   Cells exposed to test compound, solvent or positive controls for:
   3 hours (nonactivated)
   3 hours (activated)

2. Preliminary Cytotoxicity/Cytogenetic Assays: Similar procedures were used for the preliminary assessment of cytotoxicity and for the cytogenetic assay.

(a) Treatment: At ≈44 hours after initiation, replicate cultures (two/sex), were exposed to the selected test material doses, the solvent control (DMSO), or the positive controls (Mit C or CP) in both the presence and absence of S9-activation. At the end of treatment, cells were centrifuged and reincubated in fresh culture medium. Colcemid (final concentration, 0.4 μg/ml) was added 2 hours before the cultures were harvested at 72 hours postinitiation. Metaphase cells were collected, swollen in 0.075M KCl, and fixed in glacial acetic acid:methanol (1:3). Slides were prepared, stained with 10% Giemsa, and coded.

(b) Metaphase analysis: Two hundred metaphase cells in treatment and solvent control groups (100 cells/culture/donor) were scored for chromosome aberrations; ~100 cells/donor from one of the two replicate cultures for each positive control were also scored for aberrations. The mitotic index (MI) was determined for each treatment group from the analysis of 1000 lymphocytes/culture.

(c) Statistical methods: The percentage of cells with chromosome aberrations (excluding gaps) was evaluated using the Fisher's exact test ($p \leq 0.01$).

(d) Evaluation criteria: No criteria were provided to establish the validity of the assay or the biological significance of the results.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Test: The test material was reported to be soluble in DMSO. Data from the initial cytotoxicity assay indicated that few, if any, metaphases were recovered from cells treated with 110 or 550 μg/ml MTI with or without S9 activation. A marked reduction in the MI was also seen at 20 μg/ml +/-S9 in cultures derived from both donors and MIs for cultures treated with 5 μg/ml +/-S9 were ≥25% lower than control MIs. Findings for the low dose (1 μg/ml +/-S9) were comparable to the controls. Based on these results, the cytotoxicity test was repeated. Doses used in this subsequent assay ranged from 1 to 20 μg/ml +/-S9. Results from Trial 2 indicated that no metaphases were found in female donor cell cultures at the highest nonactivated dose (20 μg/ml), and the MI at this level in the presence of S9 activation was reduced by ≈60% compared to the solvent control. For male donor cells, MIs at 20 μg/ml +/-S9 were ≈60% of control. Data from the 10 μg/ml treatment groups showed an ≈30% reduction in MIs for the female donor cells both with and without S9 activation. At this level, no adverse effects on male donor cells were noted in the presence of S9 activation but an ≈32% reduction was observed without S9 activation. For the remaining doses, mitotic activity in all treatment groups was generally ≥75% of control.

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2. Cytogenetic Assays:

(a) Nonactivated conditions: Based on the evidence that lymphocytes derived from the female donor were more sensitive to the non-activated test material, the three doses selected for chromosome analysis were 2, 10, and 20 μg/ml (male) and 1, 5, and 10 μg/mL (female). MIs for lymphocyte cultures from both donors in the high-dose group were moderately reduced compared to the solvent control. Data from individual donors further indicated that the three nonactivated levels were not clastogenic in the female donor cells. However, a significant ($p<0.01$) increase in the percentage of aberrant cells was observed in the cultures derived from the male donor. The analysis of additional metaphases (100/culture) from the male donor cell cultures dosed with 10 or 20 μg/ml confirmed the initial findings and further showed a significant effect ($p<0.01$) at the high dose (Table 1). The predominant types of scored aberrations included breaks, fragments and minutes.

(b) S9-activated conditions: Doses selected for chromosome analysis in the presence of S9 activation were 2, 10, and 20 μg/ml (both donors). In agreement with the nonactivated results, MTI induced a significant ($p<0.01$) clastogenic effect in the male donor lymphocytes but only at the high dose. However, in the cultures from the female donor, genotoxic effects were significant ($p<0.01$) and dose related at 10 and 20 μg/ml. Also in agreement with the data from the nonactivated phase of testing, chromosomal damage primarily consisted of breaks, fragments, and minutes (Table 2).

From the overall findings, the study authors concluded that nonactivated and S9-activated MTI was clastogenic in cultured human lymphocytes.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study authors' interpretation of the data was correct. MTI both in the presence and absence of S9 activation produced significant ($p<0.01$) and reproducible increases in the yield of cells with abnormal chromosome morphology. The study, therefore, provides acceptable evidence of a positive response in this test system.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated July 10, 1990).

TABLE 1. Results from the Nonactivated Human Lymphocyte In Vitro Cytogenetic Assay with MTI

Substance	Dose/mL	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	Aberrations per cell[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control							
Dimethyl sulfoxide	5 µl	400[c]	10.3	2	0.005	0.25	1B;1F-M
	5 µl	200[d]	7.8	2	0.020	2.00	2B;2F-M
Positive Control[e]							
Mitomycin C	1.0 µg -	100[c]	4.4	87	1.330	70.00*	57B;8F-M;2I;20 OT
	1.0 µg -	100[d]	2.7	103	1.790	79.00*	61B;19F-M;2I;21 OT
Test Material							
MTI	2.0 µg	200[c]	7.7	0	0.000	0.00	--
	10.0 µg	400	7.0	16	0.043	3.75*	7B;7F-M;1I;1 OT
	20.0 µg	351	6.2	16	0.068	3.70*	7B;9F-M
	1.0 µg	200[d]	8.1	3	0.015	1.50	3B
	5.0 µg	200	6.7	0	0.000	0.00	--
	10.0 µg	200	5.2	2	0.010	1.00	2B

[a]Gaps excluded.
[b]Abbreviations used:

B = Break I = Interchanges
F-M = Fragments and minutes OT = Others, rearrangements

[c]Lymphocytes obtained from male donor.
[d]Lymphocytes obtained from female donor.
[e]Values were determined from a single culture/donor.

*Significantly higher ($p<0.01$) than the solvent control by Fisher's exact test.

Note: Data were extracted from the study report pp. 22, 31, and 32.

TABLE 2. Results from the S9-Activated Human Lymphocyte In Vitro Cytogenetic Assay with MTI

Substance	Dose/mL	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	Aberrations per cell[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control							
Dimethyl sulfoxide	5 µl	200[c]	6.3	2	0.010	1.00	2B
	5 µl	200[d]	7.3	0	0.000	0.00	--
Positive Control[e]							
Cyclophosphamide	50 µg	100[c]	2.6	46	0.720	39.00*	27B;9F-M;1I;9 OT
	50 µg	100[d]	1.9	49	0.750	44.00*	31B;9F-M;9 OT
Test Material							
MTI	2.0 µg	200[c]	5.8	2	0.010	1.00	1B;1F-M
	10.0 µg	200	7.1	2	0.010	1.00	2B
	20.0 µg	200	3.6	57	0.415	23.00*	37B;12F-M;2M;1I; 5 OT
	2.0 µg	200[d]	6.1	2	0.010	1.00	1B;1F-M
	10.0 µg	200	5.2	6	0.030	3.00*	6B
	20.0 µg	200	3.0	19	0.110	7.50*	11B;6F-M;2 OT

[a]Gaps excluded.
[b]Abbreviations used:

B = Break I = Interchanges
F-M = Fragments and minutes OT = Others, rearrangements
M = Multiple damage

[c]Lymphocytes obtained from male donor.
[d]Lymphocytes obtained from female donor.
[e] Values were determined from a single culture / donor.

*Significantly higher ($p<0.01$) than the solvent control by Fisher's exact test.

Note: Data were extracted from the study report pp. 23, 33, and 34.

011613

MTI MICRONUCLEUS

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll Signature: Nancy E. McCarroll
Review Section III
Toxicology Branch II/7509C Date: 4/6/95

EPA Section Head: James N. Rowe, Ph.D. Signature: James N. Rowe
Review Section III
Toxicology Branch II/7509C Date: 4/6/95

DATA EVALUATION REPORT

STUDY TYPE: In vivo micronucleus assay in mice

Tox Chem. Number:

PC CODE:

MRID Number: 431387-29

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYMS: MTI

STUDY NUMBER(S): CTL/P/3132

SPONSOR: Zeneca Inc. Wilmington, DE

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: 2-Methyl-4,5-Trimethylene-4-Isothiazolin-3-one (MTI): An Evaluation in the Mouse Micronucleus Test

AUTHORS: K. Jones and J. M. Mackay

REPORT ISSUED: October 15, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No. 431387-29), groups of five male and five female C57BL/6 mice received single oral gavage administrations of 85 or 136 mg/kg (males) or 103 or 164 mg/kg (females) MTI delivered in physiological saline. At 24, 48 or 72 hours post-exposure, high dose animals were sacrificed and bone marrow cells were examined for micronucleated polychromatic erythrocytes (MPEs). Bone marrow cells were harvested from animals in the low-dose groups only at 24 hours after treatment.

Unscheduled deaths occurred in five high-dose males and four high-dose females; dead animals were replaced with mice from a secondary group. A significant ($p<0.05$) decrease in the ratio of normochromatic to polychromatic

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erythrocytes (PCEs), indicating cytotoxic effects on the target organ, was seen in the high-dose groups (both sexes) at the 24-hour sacrifice.

A significant ($p<0.01$) increase in mean MPEs for males administered 136 mg/kg at 24 hours prompted the study investigators to evaluate an additional 2000 PCEs/male from the 24- and 48-hour sacrifice groups for the high dose and from the 24-hour sacrifice group for the low dose. Based on this expanded analysis, the data showed nonsignificant increases in MPEs for high-dose males at both sacrifice times. A similar response was present in high-dose females at 48 hours. The study authors attributed the increase in males (48 hr.) to a single animal having high MPE counts (14/1000--initial count; 13/1000 and 12/1000 --subsequent extended counts). However, increases in the number of micronuclei were seen in individual animals as follows:

2 high-dose males (24 hrs)--4.3 or 4.0 MPEs/1000 PCEs vs. 2.1 MPEs/1000 PCEs (controls)
2 high-dose males (48 hrs)--3.3 or 13.0 MPEs/1000 PCEs vs. 1.7 MPEs/1000 PCEs (control)
1 low-dose male (24 hrs)--4.7 MPEs/PCEs vs. 2.1 MPEs/1000 PCEs (controls)
1 high-dose female (48 hrs)--10 MPEs/1000 PCEs vs. 1.8 MPEs/1000 PCEs.

Although the statistical significance of the results was lost owing to the wide variability in the data, the increased sample size (3000 PCEs/males at all doses and sacrifice times) increases our confidence in the test system's ability to predict a doubling over background as a true biological effect. It is also of note that MTI induced an unambiguous clastogenic response in cultured human lymphocytes (see MRID No.431387-30). It is concluded, therefore, that the results in this mouse micronucleus assay are consistent with a weak clastogenic and/or aneugenic response.

CLASSIFICATION: Acceptable.

The study is classified as acceptable. It satisfies the guideline requirements for a micronucleus assay (84-2).

A. MATERIALS:

1. Test Material: MTI

Description: Buff colored solid
Identification Number: Ref No. MTI BP1
Purity: 95.0%
Receipt date: Not stated
Stability: Unspecified in this report but listed with an expiration date of February 28, 1991 in an accompanying mutagenicity study report (See MRID No. 431387-31)
Contaminants: None listed
Vehicle used: Physiological saline
Other provided information: The test material was stored in the dark at room temperature and prepared as a dispersion in the vehicle. The frequency of dose suspension preparation was not reported; suspensions were not adjusted to 100% a.i. or verified analytically.

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2. Control Materials:

Negative/Route of administration: None

Vehicle/Final concentration/Route of administration: Physiological saline at a dosing volume of 10 ml/kg was administered orally.

Positive/Final concentration/Route of administration: Cyclophosphamide (CP) was dissolved in physiological saline and administered orally at a dose of 65 mg/kg.

3. Test Compound:

Route of administration: Oral

Dose levels used:

(a) Range-finding Tests:
Initial trial: 50, 100, and 200 mg/kg (2 males/dose)
Repeat trial: 100, 130, 160, and 200 mg/kg (5 males and 5 females per dose) and 250 mg/kg (5 females)

(b) Micronucleus assay: 85 or 136 mg/kg (males)
103 or 164 mg/kg (females)

4. Test Animals:

(a) Species mouse Strain C57BL/6JfBL10/Alpk Age 10-14 weeks
Weight range: At dosing 20.0-29.4 g (males), 17.5-24.3 g (females)

Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals

(b) No. animals used per dose:

(1) Range-finding tests:
Initial trial: 2 males
Repeat trial: 5 males; 5 females

(2) Micronucleus assay: 15 males; 15 females (high dose groups and vehicle control group)
5 males; 5 females (low dose and positive control groups)

Note: For the micronucleus assay, an additional group of five males and five females received the respective high dose and were used as replacement animals in the event of deaths in the primary high-dose groups.

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(c) Properly maintained? YES

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound: High dose
Dosing: __x__ once _____ twice (24 hr apart)
__N/A__ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr __x__ 48 hr __x__ 72 hr

(b) Test compound: Low dose
Dosing: __x__ once _____ twice (24 hr apart)
__N/A__ other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr _____ 48 hr _____ 72 hr

(c) Vehicle control:
Dosing: __x__ once _____ twice (24 hr apart)
__N/A__ other (describe): _____
Sampling (after last dose): __x__ 24 hr __x__ 48 hr
__x__ 72 hr

(d) Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
__N/A__ other (describe): _____
Sampling (after last dose): __x__ 24 hr _____ 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

__x__ bone marrow __N/A__ others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: __1000__
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: __1000__

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the high doses of the test material or the vehicle control, the appropriate groups of animals were sacrificed by asphyxiation and cervical dislocation. Sacrifice time for the low-dose groups and positive control group was 24 hours. Bone marrow cells were collected from the femurs by dipping a fine paint brush wetted with albumin into the bone marrow canal. Recovered cells were spread onto slides and slides were stained with polychrome methylene blue and eosin and scored. Brushes were rinsed in saline between animals of the same group and separate brushes were used between groups. Slides were coded prior to scoring.

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4. Statistical Methods: The data were evaluated for statistical significance using a one-sided Student's t-test.

5. Evaluation Criteria: No criteria were provided to establish the validity of the assay or the biological significance of the results.

C. REPORTED RESULTS:

1. Range-Finding Tests: In the initial toxicity test, groups of two male mice were administered single oral doses of 50, 100, or 200 mg/kg MTI and were observed over a 4-day period. No males survived treatment with the high dose. Based on these findings, the toxicity test was repeated. In the repeat toxicity test, groups of five male and five female mice received single oral doses of 100, 130, 160, or 200 mg/kg; an additional group of females was dosed with 250 mg/kg of the test material. No deaths occurred in either sex treated with 100 or 130 mg/kg; one male in the 160-mg/kg group died. All males and 3 of 5 females receiving 200 mg/ml died; 4 of 5 females exposed to 250 mg/kg also succumbed to treatment. Median lethal doses (MLD), calculated for the 4-day observation period, were 170 mg/kg (males) and 205 mg/kg (females). From these data, the doses selected for further study in male and female mice were estimated to be 50 and 80% of the respective MLD. Accordingly, treatment levels of 85 and 136 mg/kg (males) and 103 and 164 mg/kg (females) were evaluated in the micronucleus assay.

2. Micronucleus Assay:

(a) Animal observations: The report stated that adverse reactions to treatment with the high doses were seen in both sexes throughout the study. Four high-dose males were found dead and one was sacrificed *in extremis* ≈24 hours postdosing. Similarly, two high-dose females were found dead ≈24 hours postdosing and two were sacrificed *in extremis* at ≈29 hours. Other signs of compound toxicity noted in the high-dose groups included subdued nature (both sexes) and abnormal respiratory noise in the males. Mice from the secondary group were used to replace the dead animals. No compound effects were reported for the low-dose groups.

(b) Micronucleus assay: At the 24-hour harvest, there was a significant ($p<0.5$) decrease in the PCE/NCE ratio for high-dose males and females compared to the corresponding vehicle control values. These results support an effect on hematopoiesis. Data from the analysis of PCEs for micronuclei indicated that a significant ($p<0.01$) increase in MPEs was obtained in high-dose males at 24 hours. Although the incidence of MPEs at the 48-hour sacrifice time for high-dose males was ≈3-fold higher than control, the value was not significant presumably because of the high standard deviation. MPE frequencies were not significant in the high-dose females at any sacrifice time or when the data from the high-dose exposures for each sex were combined. However, our reviewers noted the slight increase in mean MPEs in females sacrificed 48 hours after receiving 164 mg/kg MTI. A non-

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significant elevation in MPEs was also seen in the low-dose males at the 24-hour harvest; however, possible effects at later sampling times could not be assessed since later postexposure groups were not included for the low doses.

Owing to the significantly increased number of MPEs in males treated with 136 mg/kg MTI (24-harvest), an additional 2000 PCEs per male were scored in the high exposure group (24 and 48 harvest times), the low-dose group (24 hours only) and the vehicle and positive control groups.

Results from the expanded examination of bone marrow cells harvested from the male mice administered the two doses of MTI were not significant (Table 1). Combining the data from the initial and expanded analyses (3000 PCEs/male) also revealed no significant differences from the corresponding vehicle control. However, the previously noted increased incidence of MPEs in the 48-hour posttreatment high-dose males (≈2.5-fold higher than control) was still apparent when the data were combined. The study authors stated that this increase resulted from one sick animal showing MPE counts of 14, 13 and 12/1000 PCEs and that "the observation of an increase in micronucleated polychromatic erythrocytes in one animal that was very sick is not considered to be simply attributable to a chemical clastogenic effect." Our review of the individual animal data indicated that the distribution of micronuclei in the vehicle control males at 24 and 48 hours was relatively uniform , averaging 6.2 and 5.0 MPEs/animal, respectively. By contrast, high dose males at 24 and 48 hours had average MPEs of 9.6 and 12.6/animal. Similarly, two males at each sacrifice time had MPE counts that were ≈2-fold higher than the corresponding vehicle control group. In females, average MPEs/animal in the treatment groups were comparable to the control but one high-dose female sacrificed at 48 hours had 10 MPEs as compared to an average of 2.6 for the control animals. Our reviewers contend, therefore, that the observed increase in MPEs was attributable to more than a single animal. Nevertheless, the study authors concluded that MTI was not clastogenic in this micronucleus assay.

3. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess in contrast to the study authors, that the results of this mouse micronucleus assay are consistent with a weak clastogenic and/or aneugenic response. Increases in the number of micronuclei were seen in individual animals as follows:

2 high-dose males (24 hrs)--4.3 or 4.0 MPEs/1000 PCEs vs. 2.1 MPEs/1000 PCEs (controls)
2 high-dose males (48 hrs)--3.3 or 13.0 MPEs/1000 PCEs vs. 1.7 MPEs/1000 PCEs (control)
1 low-dose male (24 hrs)--4.7 MPEs/PCEs vs. 2.1 MPEs/1000 PCEs (controls)
1 high-dose female (48 hrs)--10 MPEs/1000 PCEs vs. 1.8 MPEs/1000 PCEs.

Although the statistical significance of the results was lost owing to the wide variability in the data, the increased sample size (3000 PCEs/males at all doses and sacrifice times) increases our confidence in the test system's ability to predict a doubling over background as a true biological effect. Classification of MTI as positive in this whole animal system is also supported by the clear evidence of a clastogenic effect in cultured human lymphocytes with the test material (see MRID No. 431387-30). The study satisfies the guideline requirements for a micronucleus assay.

4. QUALITY ASSURANCE MEASURES: A quality assurance (QA) statement, signed and dated October 10, 1990, was present.

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TABLE 1. Representative Results of the Micronucleus Assay in Mice Treated with MTI

Substance	Dose per kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group[b]	Mean Number of MPEs per PCEs±SD	Average Percent PCE/NCE
Vehicle Control								
Physiological saline	10 mL	24	M	5	15,000[c]	31	2.1±1.4	41.2
		48	M	5	15,000[c]	25	1.7±0.8	39.3
		72	M	5	5,000	18	3.6±1.8	33.8
		24	F	5	5,000	9	1.8±1.3	39.1
		48	F	5	5,000	13	2.6±1.7	32.4
		72	F	5	5,000	4	0.8±0.5	34.6
Positive Control								
Cyclophosphamide	65 mg	24	M	5	15,000[c]	306	20.4±9.7**	26.3**
		24	F	5	5,000	134	26.8±9.4**	35.7
Test Material								
MTI	85 mg	24	M	5	15,000[c]	42	2.8±1.3	33.9
	136 mg[d]	24	M	5	15,000[c]	48	3.2±0.9*	31.1*
		48	M	5	15,000[c]	63	4.2±5.0	34.7
		72	M	5	5,000	13	2.6±1.1	47.9
	103 mg	24	F	5	5,000	9	1.8±2.2	36.6
	164 mg[d]	24	F	5	5,000	7	1.4±0.9	29.0*
		48	F	5	5,000	17	3.4±3.9	30.8
		72	F	5	5,000	12	2.4±2.1	43.4

[a]Time after compound administration
[b]Values were calculated by our reviewers.
[c]Due to significant increases in MPEs in males at the high dose, an additional 2000 PCEs/male were scored in treatment and control groups.
[d]Five high-dose males and four high-dose females died prior to the scheduled sacrifice. Animals in the secondary group were used to replace dead animals.
[e]Results were significant ($p<0.01$) based on the count of 5,000 PCEs/group.

*Significantly different than the vehicle control ($p<0.05$) by one-sided Student's t-test.
**Significantly different than the vehicle control ($p<0.01$) by one-sided Student's t-test.

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

Data were extracted from the study report pp. 22, 23, 25, 27, and 28.

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MTI

SALMONELLA/E. COLI

EPA Reviewer: Nancy E. McCarroll
Review Section III,
Toxicology Branch II/(7509C)
Signature: Nancy E. McCarroll
Date: 3-25-95

EPA Reviewer: James N. Rowe, PhD.
Review Section III,
Toxicology Branch II/(7509C)
Signature: James N Rowe
Date: 3/28/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium/Escherichia coli/mammalian microsome gene mutation assay

TOX CHEM. NUMBER:

PC CODE:

MRID NUMBER: 431387-28

TEST MATERIAL: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one

SYNONYM(S): MTI

STUDY NUMBER(S): CTL/P/2844

SPONSOR: Zeneca Inc., Wilmington, DE

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK

TITLE OF REPORT: 2-Methyl-4,5 Trimethylene-4-Isothiazolin-3-one: An Evaluation of Mutagenic Potential Using S. typhimurium and E. coli

AUTHOR: Calander, R. D. and Priestley, K. P.

REPORT ISSUED: June 7, 1990

CONCLUSIONS--EXECUTIVE SUMMARY:

In the initial microbial/mammalian microsome plate incorporation assay (MRID No. 431387-28), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, and Escherichia coli strain WP2uvrA pKM101 were exposed to 0.32, 1.6, 8.0, 40, 100, or 200 µg/plate of MTI in the presence or absence of S9 activation derived from Aroclor 1254 induced rat liver. For the repeat trial, nonactivated levels of 0.16, 0.8, 4.0, 20, 50, or 100 µg/plate were assayed with the Salmonella strains and 0.064, 0.32, 1.6, 8.0, 20, or 50 µg/plate were assayed with E. coli. Doses evaluated under S9-activated conditions were similar to those used in the initial trial. Dimethyl sulfoxide was used as the solvent.

Although slight but significant and dose-related increases in revertant colonies of strain TA 1535 were seen in the S9-activated phase of the initial assay, the effect was not reproducible and, therefore, did not provide sufficent evidence of a mutagenic response. **MTI was cytotoxic (≥100 µg/plate - S9; 200 µg/plate +S9) in all strains but failed to induce a reproducible mutagenic effect.** All strains responded to the mutagenic action of the appropriate positive control.

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CLASSIFICATION: This study is classified as acceptable. It satisfies the guideline requirement for a gene mutation study (84-2).

A. MATERIALS:

1. Test Material: 2-Methyl-4,5-trimethylene-4-isothiazolin-3-one (MTI)

Description: Buff colored solid
Identification Number: Batch Ref No.: MTI BP1
Purity: 95.0%
Receipt date: Not reported
Stability: Test material was reported to be stable under the conditions of use.
CAS number: Not reported
Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The frequency of test material solution preparation was not provided; the report indicated that " fresh stock solutions and dilutions were prepared as necessary". Analytical determinations were not performed to verify actual concentrations used in the study; solutions were not adjusted for 100% a.i.

2. Control Materials:

Negative control: Culture medium

Solvent/final concentration: DMSO/100 µl per plate

Positive:

Nonactivation:		
N-Methyl-N'-nitro-N-nitrosoguanidine	1.0, 2.0,,5.0	µg/plate TA1535, TA100
	0.5, 1.0, 2.0	µg/plate WP2 uvrA pKM101
Daunomycin	0.2, 0.5, 1.0	µg/plate TA98
4-Nitro-o-phenylene-diamine	1.0, 2.0, 5.0	µg/plate TA1538
ICR-191	0.5, 1.0, 2.0	µg/plate TA1537
Activation:		
2-Aminoanthracene (2-AA)	1.0, 2.0, 5.0	µg/plate WP2 uvrA pKM101
	0.5, 1.0, 2.0	µg/plate TA1535, TA1537
	0.2, 0.5, 1.0	µg/plate TA1538, TA98, TA100

3. Activation: S9 derived from male Alderley Park (Alpk:APfSD)

__x__ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

Three batches of S9 homogenate, prepared by the performing laboratory, were used in the study. The composition of the S9 mix was as follows:

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S9 mix composition:

Component / Concentration :

100 mM Sodium phosphate buffer
5 mM Glucose 6-phosphate
4 mM NADP
8 mM $MgCl_2$
33 mM KCl
10% S9

4. Test Organism Used: S. typhimurium strains
____ TA97A __x__ TA98 __x__ TA100 _____ TA102 _____ TA104
__x__ TA1535 __x__ TA1537 __x__ TA1538
list any others: E. coli strain WP2uvrA pKM101

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

a. Preliminary Cytotoxicity Test: Six doses ranging from 1.6 to 5000 µg/plate were assayed with and without S9 activation using strain TA100. Triplicate plates were prepared per dose per condition.

b. Mutation Assays:

Initial Trial: Six levels (0.32, 1.6, 8.0, 40, 100, and 200 µg/plate +/-S9) were evaluated in all strains. Triplicate plates were used per strain, per dose, per condition.

Repeat Assay: For the repeat assay, nonactivated concentrations ranging from 0.16 to 100 µg/plate were tested in S. typhimurium and 0.064 to 50 µg/plate were tested with E. coli. Doses evaluated under S9-activated conditions were similar to those used in the initial trial.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
__x__ Standard plate test
_____ Pre-incubation (_____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

2. Protocol:

(a) Preliminary cytotoxicity /mutation assays: Similar procedures were used for the preliminary assessment of cytotoxicity and the mutation assays. Bijou bottles were prepared to contain: 0.1 mL of an overnight broth culture of the appropriate tester strain, 0.5 ml of the S9 mix cofactor buffer or the S9 mix, and 0.1 ml of the appropriate test material dose, solvent, or positive control. To each prepared bottle, a 2.0-mL volume of top agar supplemented with histidine / biotin for the S. typhimurium strains or tryptophan for the E. coli strain was added. The contents of the

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bottles were mixed, poured over Vogel-Bonner minimal medium and incubated at 37°C for 3 days. At the end of incubation, plates were examined for the presence of a background lawn of growth and revertant colonies were counted. Means and standard deviations were determined from the counts of triplicate plates per strain, per dose, per condition for the test material. Five plates per strain, per condition were used for the solvent control (DMSO) and duplicate plates per strain, per condition were prepared for the negative control and each of the three concentrations of the positive controls.

(b) Evaluation criteria:

Assay validity: The assay was considered valid if: (1) the concurrent solvent control data were acceptable; (2) the positive control data showed clear positive responses and (3) at least the two lowest test material doses were not cytotoxic.

Positive response: The test material was considered positive if it induced a reproducible, statistically significant and dose-related increase in the number of revertant colonies relative to the corresponding solvent control or a significant ≥2-fold increase in revertant colonies at one or more doses.

(c) Statistical methods: The data were evaluated for statistical significance ($p<0.01$) using a one-tailed Student's t-test.

C. REPORTED RESULTS

1. Preliminary Cytotoxicity Assay: Six doses of MTI (1.6-5,000 μg/plate) were assayed with or without S9 activation using S. typhimurium strain TA100. No cell survived exposure to levels ≥ 200 μg/plate with or without S9. The remaining levels (1.6, 8.0 or 40 μg/plate +/- S9) were not cytotoxic. Based on these finding, doses chosen for the initial mutation assay ranged from 0.32 to 200 μg/plate +/-S9.

2. Mutation Assays:

Initial Assay: In the first trial, MTI was severely cytotoxic in all S.typhimurium strains at levels ≥ 100 μg/plate -S9 and at 200 μg/plate +S9. The test material was also severely cytotoxic in E. coli WP2 uvrA pKM101 at nonactivated doses ≥40 μg/plate and S9-activated levels ≥100 μg/plate. As shown in Table 1, slight but significant and dose-related increases in his+ colonies of TA 1535 were seen at all noncytotoxic levels in the S9-activated phase of testing. The peak response (≈1.8-fold increase) was noted at 8.0 and 40 μg/plate. For the remaining strains, the sporadic significant increases in colony counts were not considered to be indicative of a mutagenic effect. Based on the overall findings, the assay was repeated.

Repeat Assay: The dose ranges selected for the repeat assay were as follows: 0.16-100 μg/plate -S9; 0.32-200 μg/plate +S9--all Salmonella strains and 0.064-50 μg/plate +/-S9-- E. coli. In agreement with the earlier results, MTI was cytotoxic in all Salmonella strains at 100 μg/plate -S9 and 200 μg/plate + S9. Reductions in his+ colonies were also apparent at 50 μg/plate -S9 for all S. typhimurium strains except TA 100. The highest dose assayed with E. coli (50 μg/plate +/-S9) was neither cytotoxic nor mutagenic. Similarly, the nonactivated or S9-activated test material was not mutagenic in any Salmonella tester strain (Table 2).

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By contrast, all strains responded in a dose-related manner to the appropriate nonactivated and S9-activated positive control concentrations in both trials. From the overall findings, the study authors concluded that MTI was not mutagenic in this test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. The test material was assayed to cytotoxic levels (≥100 µg/plate -S9/ 200 µg/plate +S9) with S. typhimurium and E. coli tester strains, but failed to induce a mutagenic effect. Although slight but significant increases in revertant colonies of S. typhimurium TA 1535 were seen in the initial trial, the effect was not reproduced in the repeat assay. The inital finding is, therefore, insufficient to conclude a positive response. It was noted that the spontaneous reversion rates for strains TA1535 and TA98 were borderline acceptable, nevertheless, the response of all tester strains to the appropriate direct-acting or promutagenic positive controls indicated that the assay had an adequate level of sensitivity to detect mutagenesis. It was concluded, therefore, that MTI was negative in this microbial test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement was signed and dated June 1, 1990.)

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TABLE 1. Representative Results of the Initial Microbial/Mammalian Microsome Mutation Assay with MTI

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a] S. typhimurium TA1535	TA1537	TA1538	TA98	TA100	E. coli WP, uvrA
Solvent Control								
DMSO	100 µL	-	49.6±6.0	4.2±1.3	6.0±1.7	16.2±4.9	156.2±14.7	125.8±13.8
	100 µL	+	39.4±13.1	6.8±1.8	7.6±2.9	15.0±3.2	147.4±19.4	118.6±5.6
Positive Controls[b]								
MNNG	2 µg	-	261.5±27.6	--	--	--	445.0±151.3	930.5±81.3
ICR 191	1 µg	-	--	193.0±19.8	--	--	--	--
4NPD	2 µg	-	--	--	173.5±9.2	--	--	--
DR	1 µg	-	--	--	--	232.5±9.2	--	--
2AA	1 µg	+	145.0±14.1	44.0±9.9	567.0±186.7	699.0[e]	1629.5±197.3	220.0±19.8
Test Material								
MTI	8 µg[d]	-	80.7±7.0[c]	6.0±3.6	5.7±3.1	16.0±5.3	149.0±26.0	115.7±4.0
	40 µg	-	55.0±2.6	4.7±3.1	2.3±1.5	17.7±5.7	132.7±24.1	0.0±0.0
	1.6 µg[d]	+	68.0±9.2[c]	5.0±0.0	11.7±2.5[c]	22.3±4.6[c]	161.3±5.0	117.7±8.5
	8.0 µg	+	72.0±1.7[c]	4.7±3.8	11.7±2.5[c]	20.3±7.1	173.0±24.2	117.3±5.9
	40 µg	+	70.0±12.1[c]	5.7±2.5	11.0±5.0	21.0±7.8	157.3±10.7	100.3±11.0
	100 µg	+	64.0±6.9[c]	3.0±2.0	7.3±5.0	14.7±2.3	155.7±10.3	0.0±0.0

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses.
[b]Three levels of each positive control were assayed; results for all strains were generally significant ($p<0.01$) at the majority of doses. The presented data were selected as representative.
[c]Data are from duplicate plates; contamination was reported for the third plate.
[d]Results for lower doses (0.32 or 1.6 µg/plate -S9 or 0.32 µg/plate +S9) were not indicative of a mutagenic effect.

[e]Increases at these levels were significant but did not achieve a doubling of the background values.

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, pp. 17 and 18 and 23-27

TABLE 2. Representative Results of the Repeat Microbial/Mammalian Microsome Mutation Assay with MTI

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a] S. typhimurium TA1535	TA1537	TA1538	TA98	TA100	E. coli W_n uvrA
Solvent Control								
DMSO	100 µL	-	36.2±7.6	5.8±1.9	9.8±2.2	14.4±2.1	158.6±7.7	138.0±18.5
	100 µL	+	44.2±7.0	6.2±2.5	12.8±5.9	16.4±3.6	157.4±19.9	123.2±12.0
Positive Controls[b]								
MNNG	2 µg	-	725.5±153.4	--	--	--	1494.0±625.1	3295.0±35.4
ICR 191	1 µg	-	--	143.5±16.3	--	--	--	--
4NPD	2 µg	-	--	--	213.5±7.8	--	--	--
DR	1 µg	-	--	--	--	367.0±70.7	--	--
2AA	1 µg	+	155.5±14.8	52.5±6.4	846.5±275.1	934.0[c]	1328.0±572.8	214.0±9.9
Test Material								
MTI	20 µg[d]	-	31.3±2.1	6.7±2.1	4.3±1.2	20.0±5.3[e]	183.7±3.5[e]	123.7±15.0
	50 µg	-	4.0±6.9	0.0±0.0	1.3±2.3	14.3±5.5	137.0±17.8	122.0±13.5
	50 µg[d]	+	--	--	--	--	--	117.0±6.0
	100 µg	+	24.7±4.9	5.7±0.6	8.7±0.6	11.7±6.7	155.0±3.6	--

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses

[b]Three levels of each positive control were assayed; results for all strains were generally significant ($p<0.01$) at the majority of doses. The presented data were selected as representative.

[c]One plate was reported to be contaminated, presented data are from the counts of duplicate plates.

[d]Results for lower doses (0.16, 0.8, or 4.0 µg/plate -S9--S. typhimurium or 0.064, 0.32, 1.6, or 8.0 µg/plate -S9--E.coli; and 0.32, 1.6, 8.0, or 40 µg/plate +S9--S. typhimurium or 0.064, 0.32, 1.6, 8.0, or 20 µg/plate +S9--E. coli) did not suggest a mutagenic effect.

[e]Increases at these levels were significant but did not achieve a doubling of the background values.

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, pp. 19-22 and 28-31.